1978

Part I. A Specific Spectrophotometric Determination of Long Chain Nitro Compounds. Part II. A Specific Spectrophotometric Determination of Ozone in Air in the Presence of Nitrogen Oxides.

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PART I

A SPECIFIC SPECTROPHOTOMETRIC DETERMINATION OF LONG CHAIN NITRO COMPOUNDS

PART II

A SPECIFIC SPECTROPHOTOMETRIC DETERMINATION OF OZONE IN AIR IN THE PRESENCE OF NITROGEN OXIDES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Chemistry

by
Randle Scott Collard
B.S., Trinity University, 1973
December 1978

ACKNOWLEDGEMENTS

I would like to express my thanks to the following:

Dr. William A. Pryor for his guidance and support,

Dr. Daniel F. Church for his helpful discussions and advice,

the Charles E. Coates Memorial Fund, donated by Dr. George H. Coates and administered by the L.S.U. Foundation, for financial assistance in the preparation of this dissertation,

the National Institute of Health for financial assistance,

my parents for their support, both tangible and intangible,

and most of all, my wife, without whom this would not have been possible (Proverbs 31:10-12 and 25-31).

FOREWORD

This dissertation is comprised of two independent and unrelated projects: Part I: A Specific Spectrophotometric Determination of Long Chain Nitro Compounds and Part II: A Specific Spectrophotometric Determination of Ozone in Air in the Presence of Nitrogen Oxides.

In the interests of clarity and convenience, the dissertation is, therefore, also divided into two parts. While the Table of Contents, the list of References, and the lists of Tables and Figures for the two parts are combined, the text for each part is separate. The Introduction for Part I begins on page 1, and the Introduction for Part II on page 56.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS ii

FOREWORD iii

TABLE OF CONTENTS iv

LIST OF TABLES xii

LIST OF FIGURES xiii

LIST OF ABBREVIATIONS AND SYMBOLS xv

ABSTRACT xvii

PART I

INTRODUCTION 1

Effects of Nitrogen Dioxide 1

Lung Tissue Model Systems 1

Possible Mechanisms for the Autoxidation of PUFA by ppm NO_2 1

Need for a Method for the Determination of Nitro-β-hydroperoxides 7

Restrictions and Interferences 7

REVIEW OF LITERATURE METHODS FOR THE DETERMINATION OF THE NITRO-β-HYDROPEROXIDES 8

DETERMINATION AS NITRO 8

Redox Determinations 8

Total Nitrogen Determinations 10

Nitrous Acid Methods 10

TABLE OF CONTENTS - Continued

	Page
Aci Ion Methods	10
Acid or Base Reagent Methods	12
Spot Test Methods	12
DETERMINATION BY CONVERSION TO NITRITE ION	12
Methods Using Acid	12
Methods Using Base (The Sweet Method)	12
DEVELOPMENT OF A DETERMINATION METHOD	15
REDUCTION OF NITRO COMPOUNDS TO AMINES	15
Metal Catalyzed Reductions	15
Hydrosiloxane Reductions	15
DETERMINATION OF ALCOHOL-AMINE	17
Alcohol Interference	17
Imine Determination Methods	17
Spot Test Methods	17
Indicator Dye Methods	17
The Methyl Orange Method	18
MATERIALS AND EQUIPMENT	20
EXPERIMENTAL PROCEDURES	23
NITRITE DETERMINATIONS	23
The Sweet Method	23
The Estes and Baughman Method	23

TABLE OF CONTENTS - Continued

	Page
POLYMETHYLHYDROSILANE REDUCTION	24
METHYL ORANGE AMINE DETERMINATIONS	25
Original Silverstein Method	25
Adapted Methyl Orange Method	26
LONG CHAIN ALIPHATIC NITRO COMPOUND DETERMINATION METHOD	26
SYNTHESIS OF PRIMARY NITRO COMPOUNDS	27
Reaction of Primary Halides with Sodium Nitrite	27
ATTEMPTED SYNTHESIS OF SECONDARY NITRO ESTER	28
Methyl Oleate + HBr	28
Methyl 9 or 10-Bromooctadecanoate + Sodium Nitrite	28
Identification and Separation Attempts	29
Crown Ether Catalysis	29
Tetralkyl Ammonium Catalysis	29
ATTEMPTED SYNTHESIS OF SECONDARY NITRO COMPOUND	30
Synthesis of n-Butyl Tosylate	30
Reaction of n-Butyl Tosylate with 1-Nitrooctane	30
ATTEMPTED SYNTHESIS OF NITRO-β-HYDROPEROXIDE ESTER	30
Methyl Oleate + NO_2 (ppm) in Air	30
Methyl Oleate + N_2O_4 in Oxygen Atmosphere	31
Separation Attempts	31
ATTEMPTED SYNTHESIS OF NITRO-β-ALCOHOL	32
Esterification of 6-Bromohexanoic Acid	32
Methyl 6-Nitrohexanoate + Dodecanal (IRA 400 Resin Catalysis)	32

TABLE OF CONTENTS - Continued

Page

Methyl 6-Nitrohexanoate + Dodecanal (Sodium Methoxide Catalysis). 33

Dodecanal + Nitromethane 33

Dodecanal + 1-Nitrooctane 33

ATTEMPTED SYNTHESIS OF NITRO-β-KETONE 34

Methyl Sebacoate + 1-Nitrooctane 34

Hexanoyl Chloride + Nitromethane 34

RESULTS AND DISCUSSION 36

ATTEMPTED DETERMINATION OF NITRO-β-HYDROPEROXIDE BY CONVERSION TO NITRITE ION 36

Discovery of Error in the Literature 36

Model Compounds 36

Lipid and Hydroperoxide Interference Studies 37

The Original Estes and Baughman Procedure 38

Solvent Studies 38

Increased Reagent Concentration Studies 39

Determination of 2-Nitrooctane with the Adapted Procedure 41

Determination of Nitro-β-hydroperoxide by the Adapted Method . . 41

Conclusions 42

POLYMETHYLHYDROSILANE REDUCTION STUDIES 42

METHYL ORANGE AMINE DETERMINATION 42

Determination of Dodecyl Amine by the Adapted Methyl Orange Method 44

THE NITRO-LIPID DETERMINATION METHOD 44

TABLE OF CONTENTS - Continued

Page

Determination of Dodecyl Amine by the Nitro-Lipid Procedure . . . 44

Determination of 1-Nitrohexadecane by the Nitro-Lipid Procedure 47

Determination of Nitro-β-hydroperoxide by the Nitro-Lipid Procedure 47

Interferences 47

Determination of Other Long Chain Nitro Compounds 50

Determination of Short Chain and Polar Nitro Compounds 50

SYNTHESIS OF LONG CHAIN NITRO COMPOUNDS 50

Synthesis of a Primary Nitro Compound by S_N2 Displacement of a Primary Halide 51

Attempted Synthesis of a Secondary Nitro Compound by S_N2 Displacement of a Secondary Halide with Nitrite Ion 51

Attempted Synthesis of a Secondary Nitro Compound via the S_N2 Displacement of a Tosylate by an α-Nitro Carbanion 51

ATTEMPTED MODEL COMPOUND SYNTHESIS FOR THE STANDARDIZATION OF THE NITRO-LIPID METHOD FOR THE DETERMINATION OF NITRO-β-HYDROPEROXIDES 52

Attempted Synthesis of a Nitro-β-hydroperoxide Ester 52

Attempted Synthesis of a Nitro-β-hydroperoxide Ester Using N_2O_4 in an Oxygen Atmosphere 53

Attempted Synthesis of a Nitro-β-alcohol Ester by Coupling of an Aldehyde with an α-Nitro Ester 53

Attempted Synthesis of a Nitro-β-alcohol by Coupling of an Aldehyde and a Primary Nitro Carbanion 54

Attempted Synthesis of a Nitro-β-ketone Ester by Coupling of a Diester with a Primary Nitro Carbanion 54

Attempted Synthesis of a Nitro-β-ketone by Coupling of an Acid Chloride and a Primary Nitro Carbanion 54

TABLE OF CONTENTS - Continued

Page

PART II

INTRODUCTION 56

Standardization of Ozone Determination Methods 56

THE IODOMETRIC METHOD 57

Mechanism 58

Stoichiometric Parameters 59

Early Titrimetric Methods 60

The Saltzman Iodometric Method 61

The Ingols Iodometric pH Study 64

The Cohen, Purcell, and Altshuller Interference Study 64

The Boyd Stoichiometry Challenge 65

Refutation of Boyd and Support for the NBKI Method 65

The Iodometric Standardization Reference Method 66

The Flamm Investigation of the NBKI Method 68

OTHER DETERMINATION METHODS 70

The Ethylene Chemiluminescence Method 71

Nitrogen Dioxide Methods 71

Mast (Electrochemical) Ozone Meters 71

Miscellaneous Methods 72

OZONOLYSIS METHODS 73

Mechanism 73

Determination of Ozone by Quantification of the Carbonyl Fragment from Ozonolysis 74

TABLE OF CONTENTS - Continued

Page

The West Method (Advantages of Formaldehyde) 78

Factors Affecting the Yield of Formaldehyde from the Ozonolysis of a Terminal Olefin 81

Hammett Studies of Styrenoid System Ozonolysis 81

Formaldehyde Determination Methods 82

Standardization 83

Free Radical Inhibitors 83

MATERIALS AND EQUIPMENT 85

EXPERIMENTAL PROCEDURES 86

OZONE GENERATION AND DETERMINATION APPARATUS 86

Ozone Generator 86

Detection Apparatus 86

THE IODOMETRIC METHOD 86

Standardization of Iodine Solution 86

Preparation of a Standard Curve 88

Analysis of Air Samples 88

STANDARDIZATION OF COMMERCIAL FORMALDEHYDE 89

FORMALDEHYDE DETERMINATION METHOD 90

Preparation of Standard Curve 90

Sample Determination 91

DPE OZONE DETERMINATION PROCEDURE 91

STOICHIOMETRY STUDIES 92

TABLE OF CONTENTS - Continued

Page

INHIBITOR STUDIES 93

NO_2 INTERFERENCE STUDIES 93

RESULTS AND DISCUSSION 94

FORMALDEHYDE DETERMINATION 94

Standardization of Commercial Formaldehyde 94

Preparation of Standard Curves 94

Blank Determination 96

Color Development 96

Temperature Effects 96

STANDARDIZATION OF THE IODOMETRIC METHOD 98

THE DETERMINATION OF OZONE BY OZONOLYSIS OF A TERMINAL OLEFIN . . 98

Solvent Study 98

Olefin Study 100

Stoichiometry Study 101

Interferences 102

Inhibitor Study 102

Conclusion 104

REFERENCES: PART I 106

REFERENCES: PART II 114

VITA 120

LIST OF TABLES

Table		Page
	PART I	
I	Effect of Base Concentration on the Yield of Nitrite Ion	39
II	Effect of Peroxide Concentration on the Yield of Nitrite Ion	40
III	Percent Theoretical Yield of 2-Nitrooctane from the Adapted Sweet Procedure	41
	PART II	
I	Yield of Iodine from Various Iodometric Method Interferents (by Molar Absorptivities)	64
II	Yield of Formaldehyde from Various Terminal Olefins .	100
III	Yield of Formaldehyde from the DPE Method for a Range of Ozone Concentrations	101

LIST OF FIGURES

Figure		Page
	PART I	
I	Initiation by Abstraction	2
II	Initiation by Addition	3
III	Propagation	5
IV	Disproportionation	6
V	Termination	6
VI	Isometric Nitro-Beta-Hydroperoxides	9
VII	Pseudonitrole	11
VIII	Aci-Ion Tautome ization	11
IX	The Sweet Determination Sequence	14
X	PolyMethylHydroSilane (PMHS)	16
XI	Methyl Orange	19
XII	Percent Reaction of Nitrocyclohexane vs Time	43
XIII	Absorbance (485 nm) vs Concentration of DDA (by the Methyl Orange Method	45
XIV	Absorbance (485 nm) vs Concentration of DDA (by the Nitro-Lipid Method)	46
XV	Absorbance (485 nm) vs Concentration of 1-Nitrohexadecane	48
XVI	Absorbance (485 nm) vs Concentration of Nitro-Lipid	49
	PART II	
I	Ozone-Iodide Reaction Mechanism	58

LIST OF FIGURES - Continued

Figure		Page
II	Molozonide Formation ...	74
III	Molozonide Decomposition (Ionic)	75
IV	Molozonide Decomposition (Radical)	76
V	Criegee Ozonides	77
VI	West-Gaeke SO_2 or Reverse West-Gaeke CH_2O Method	79
VII	Mechanism of Formaldehyde-Sulfite Reaction	84
VIII	Ozone Generating Apparatus	87
IX	Absorbance (580 nm) vs Concentration of Formaldehyde	95
X	Absorbance (580 nm) vs Time	97
XI	Absorbance (352 nm) vs Concentration of I_3^-	99
XII	Concentration CH_2O vs Concentration of O_3	103

LIST OF ABBREVIATIONS AND SYMBOLS

PART I

18:1	Methyl oleate
18:2	Methyl linoleate
18:3	Methyl linolenate
PUFA	PolyUnsaturated Fatty Acid ester(s)
NED	N-1-Naphthylethylene diamine dihydrochloride
SAM	Sulfanilamide
BHT	Butylated hydroxytoluene
DMSO	Dimethyl Sulfoxide
PMHS	Polymethylhydrosilane
NMR	Nuclear Magnetic Resonance (spectrum)
IR	Infrared (spectrum)
TLC	Thin Layer Chromatography
GLPC	Las Liquid Chromatography
NBH	Nitro-β-hydroperoxide (ester)
THF	Tetrahydrofuran

PART II

NMR	Nuclear Magnetic Resonance (spectrum)
IR	Infrared (spectrum)
LC	Low Concentration ozone (0.1-5.0 μl/l)
IC	Intermediate Concentration ozone (6.0-99.0 μl/l)

HC	High Concentration ozone (99+ μl/l)
NBKI	Neutral Buffered (phosphate) Potassium Iodide method
EPA	Environmental Protection Agency
UV	Ultraviolet (spectrum)
DPE	1,1-Diphenylethylene
ppm	Parts Per Million (μg/g)

ABSTRACT

This dissertation is divided into two parts:

Part I. A Specific Spectrophotometric Determination of Long Chain Nitro Compounds.

When lung tissue model systems such as neat lipid are exposed to ppm (μg/g) nitrogen dioxide in air, free radical initiated autoxidation occurs. Initiation can occur either by addition of nitrogen dioxide to one of the lipid double bonds or by abstraction of an allylic hydrogen by nitrogen dioxide. In the former mechanism, each initiation sequence produces a molecule of nitro-β-hydroperoxide, and determination of the amount of nitro-β-hydroperoxide provides a measure of the amount of initiation occurring by addition. A determination method for these nitro-β-hydroperoxides was, therefore, developed. The method determines micromolar quantities of nitro-β-hydroperoxide or any other long chain-nonpolar nitro compound by reduction of the nitro group to an amine, using polymethylhydrosilane and Palladium on charcoal, followed by a colorimetric determination of the amine formed. The amine is protonated and allowed to react with the sulfonic acid anion of methyl orange to form a neutral salt, which is then extracted into an organic layer and determined spectrophotometrically. The method is specific for nitro compounds in the absence of compounds which complex amines or which are reduced to amines by the polymethylhydrosilane/Palladium on charcoal system. Although limited to the determination of compounds which form extractable salts with methyl orange, the method can be adapted for the

determination of short chain or polar nitro compounds simply by using a different amine method, since these compounds are also reduced to amines by the polymethylhydrosilane/Palladium on charcoal system.

Part II. A Specific Spectrophotometric Determination of Ozone in Air in the Presence of Nitrogen Oxides.

A specific spectrophotometric determination for ozone in air was developed using the only reaction specific for ozone: ozonolysis, i.e., the reaction of ozone with a double bond to produce an ozonide which cleaves to form a carbonyl fragment and a zwitterion. A survey of the literature showed that the direction of cleavage for an unsymmetrical ozonide is governed by the resonance and inductive effects of the substituents on the stabilities of the carbocationic portions of the two possible zwitterions. Several terminal olefins were, therefore, investigated in order to determine which gave the highest yield of formaldehyde from ozonolysis. DPE (1,1-diphenylethylene) was found to give the highest yield, 90% over an ozone concentration range of 0.05-5.00 μg/g of air, and was incorporated into an ozone determination procedure. In the procedure, air containing ozone is passed through an impinger containing DPE, and the formaldehyde produced is blown out of the impinger into a bubbler where it is trapped in water. After trapping is completed, the bubbler is removed and the formaldehyde is determined by reaction with sulfur dioxide, followed by reaction of the adduct produced with pararosaniline (the reverse West-Gaeke method), and spectrophotometric determination of the product formed. The method has the advantage of being much less subject to

interferences than the iodometric method, the ethylene chemiluminescence method, the UV photometric method, and several other ozonolysis methods. The carbonyl fragment produced, formaldehyde, is blown out of the ozone trap into a second trap and is thus protected from further reaction. Sulfur dioxide does not interfere, indeed it is used as one of the reagents. Lastly, interference by free radical initiators or oxidants is prevented by the addition of 2% mesitol (2,4,6-trimethylphenol) to the DPE trap; this concentration has been found to allow the determination of ozone in air containing as much as 5 μg/g of air nitrogen dioxide.

INTRODUCTION

Effects of Nitrogen Dioxide

Nitrogen Dioxide is an important air pollutant known to cause a number of physiological effects, primarily in the lung[1-5], in addition to its role in the formation of smog.

Lung Tissue Model Systems

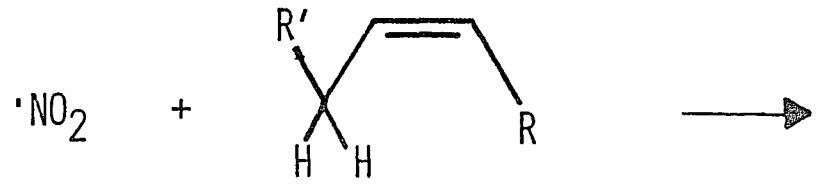
Our laboratories are currently investigating the mechanism of the nitrogen dioxide initiated, free radical autoxidation of simple models of lung tissue. In these model studies, neat lipids or Poly-Unsaturated Fatty Acid esters (PUFA) are used as the model compounds. The three lipids under investigation are methyl oleate, methyl linoleate, and methyl linolenate (18:1, 18:2, and 18:3, respectively). One ml of one of the lipids is placed in a midget impinger and is exposed to a stream of air containing nitrogen dioxide at the ppm (μg/g) level. The experimental apparatus and procedures are described in detail by Pryor, et al.[6,7]

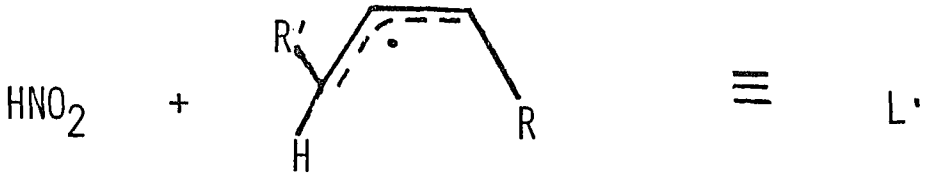
Possible Mechanisms for the Autoxidation of PUFA by ppm NO_2

Two initiation mechanisms are possible for the free radical autoxidation of PUFA by nitrogen dioxide: the abstraction of an allylic hydrogen from a PUFA molecule by NO_2 (Figure I) and addition of nitrogen dioxide to one of the double bonds of a PUFA molecule (Figure II). The

FIGURE I

Initiation by Abstraction





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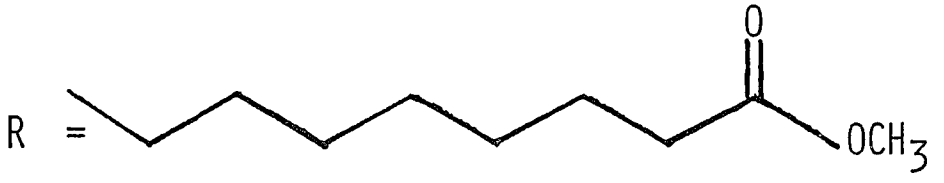


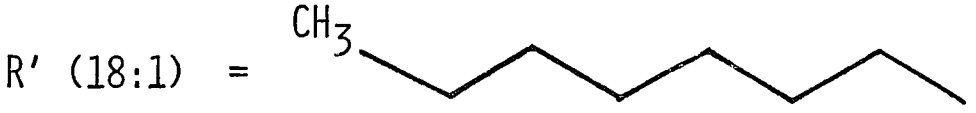
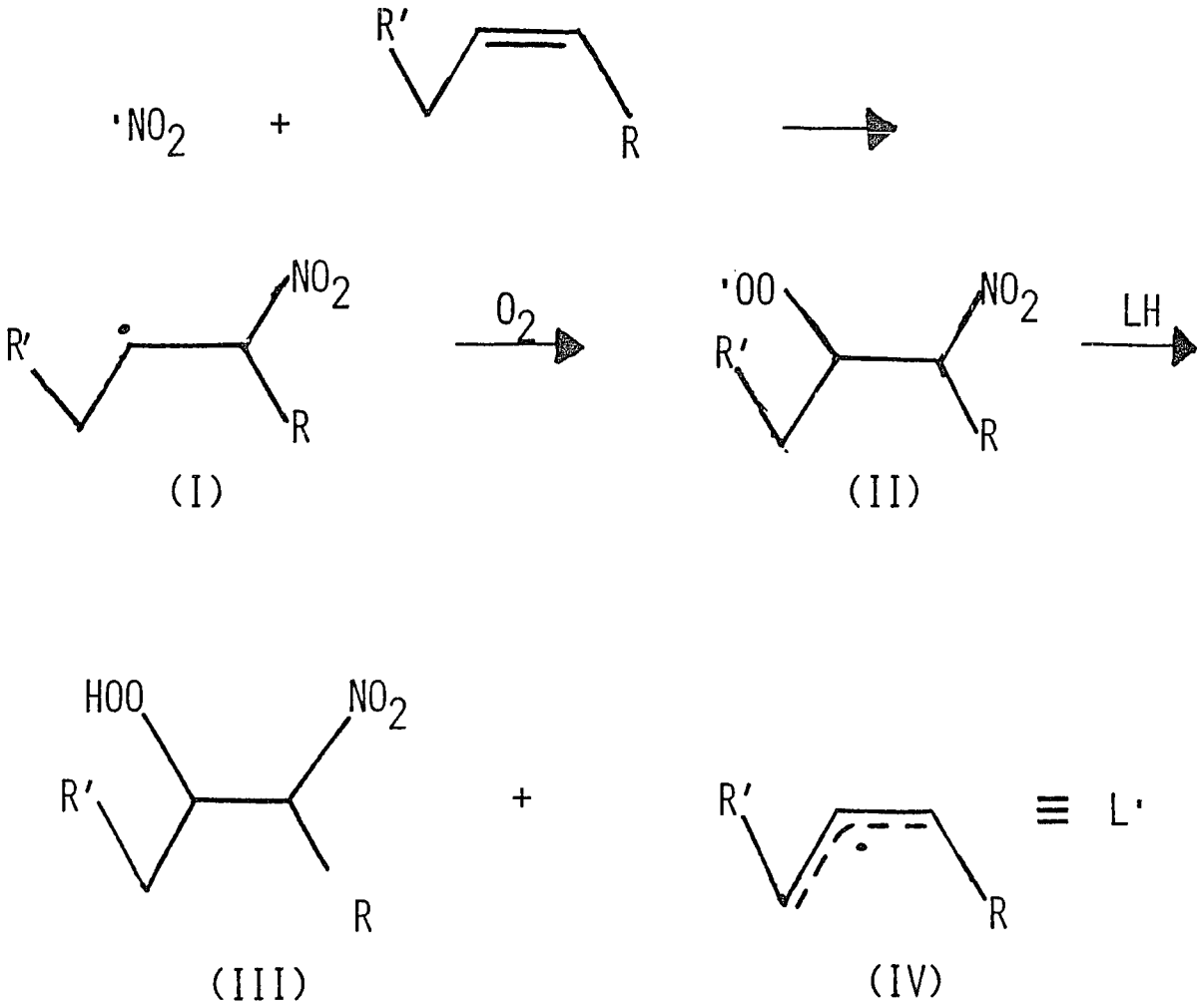


FIGURE II

INITIATION BY ADDITION



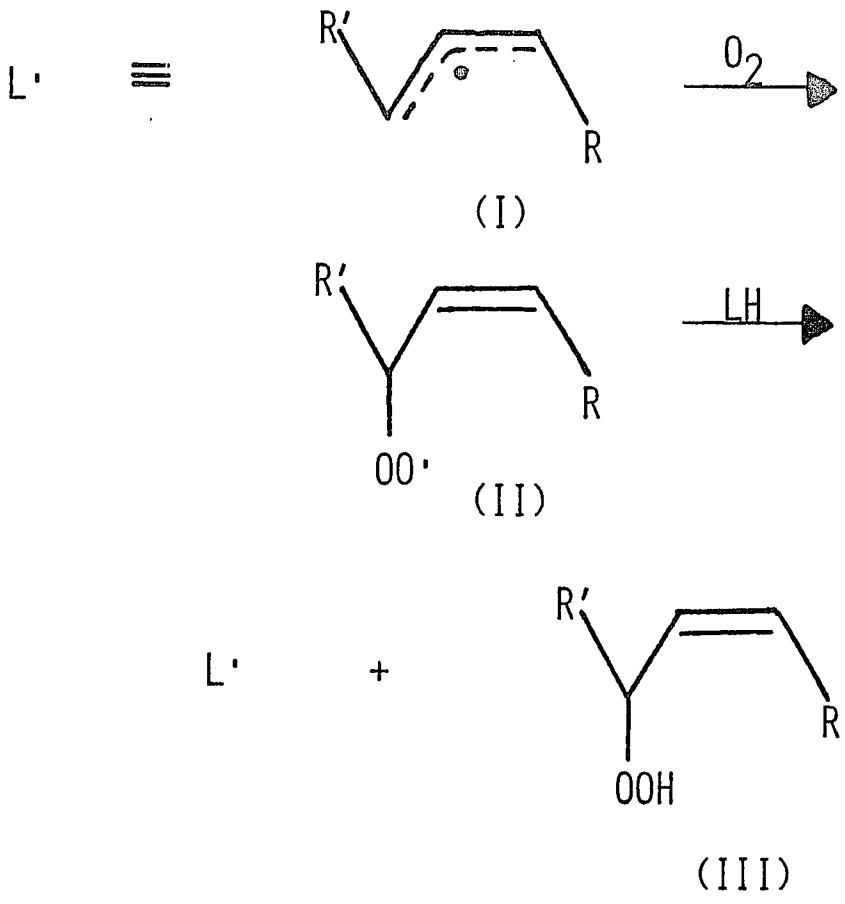
R AND R′ (SEE FIGURE I)

abstraction mechanism produces nitrous acid and a resonance stabilized lipid radical, L., (I, Figure I) which then enters the propagation steps by reaction with molecular oxygen to produce the alkyl-peroxy radical (II, Figure III). The nitrous acid formed disproportionates to produce additional nitrogen dioxide[8] (Figure IV), and the additional nitrogen dioxide, in turn, initiates other chains. When possible, i.e., for 18:2 and 18:3, the hydrogen abstracted and the lipid radical formed will be doubly allylic rather than singly allylic, since the doubly allylic hydrogens are twenty times more reactive.[8] The extent of initiation by the abstraction mechanism is presently being determined by measurement of the amount of water formed in the disproportionation step, this work is being carried out by John W. Lightsey and will be a part of his dissertation.[9]

The addition mechanism, initiation by the addition of a nitrogen dioxide molecule to one of the double bonds of a PUFA molecule (Figure II), produces a nitro-alkyl radical (I, Figure II). Since the rate constants for the reactions of oxygen and nitrogen dioxide with free radicals are both large[10] and since oxygen is present in far greater concentration than nitrogen dioxide, the nitro-alkyl radical reacts with a molecule of oxygen to give a nitro-peroxy radical (II, Figure II). This radical then abstracts a hydrogen from another lipid molecule to form the first stable product from this initiation sequence, a nitro-β-hydroperoxide (III, Figure II). The lipid radical formed, L., (IV, Figure II) then enters the propagation steps (Figure III), and several hydroperoxides are formed before the chain is terminated (Figure V). Termination proceeds almost exclusively by the reaction of two alkyl-peroxy radicals (2, Figure V). The concentration of alkyl

FIGURE III

PROPAGATION



R AND R′ (SEE FIGURE I)

FIGURE IV

DISPROPORTIONATION

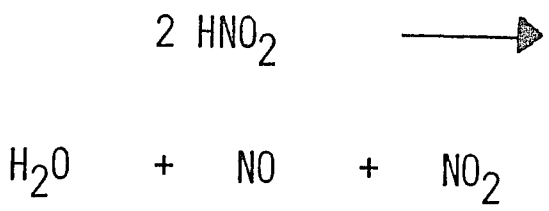
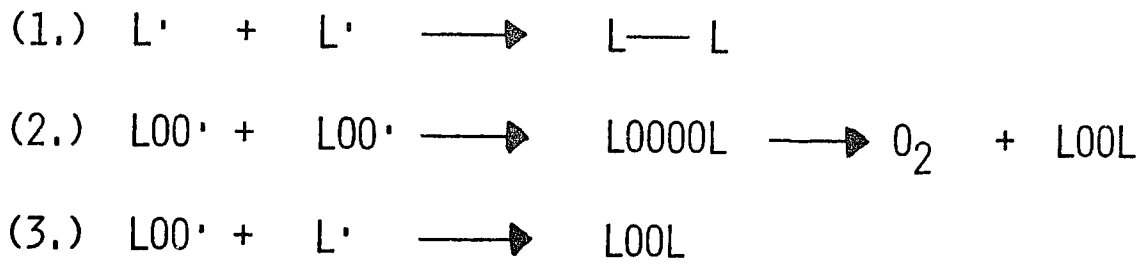


FIGURE V

TERMINATION



radicals (L·) is low due to the rapid reaction of L· with oxygen,[10] hence termination reactions involving the alkyl radical (1&3, Figure V) are not important.

Need for a Method for the Determination of Nitro-β-hydroperoxides

Since the nitro-β-hydroperoxides formed in the initiation sequence are the first stable initiation products and since one nitro-β-hydroperoxide is formed for each initiation event, a measurement of the concentration of nitro-β-hydroperoxide product provides a means for the determination of the rate of initiation by the addition mechanism. This, in turn, provides a means for determining the chain length, or the rate of appearance or disappearance of a given substance divided by the rate of initiation.[11] Since knowledge of these is essential to determining the extent of initiation by the addition mechanism, a method for the determination of the nitro-β-hydroperoxides was sought.

Restrictions and Interferences

The determination of the concentration of nitro-β-hydroperoxide in an autoxidation system is extremely difficult. First, the aliquot taken for analysis must be small (less than 100 µl), since the entire lipid sample can only be about 1 ml, in order that the reaction can be studied in a flow system.[6] Second, since the nitro-β-hydroperoxide is formed only in the initiation step and since it is necessary to allow the reaction to proceed to only about 10% completion to prevent the reactions of products from becoming important, the method must be very sensitive. Third, there are several structural isomers of the

nitro-β-hydroperoxide which are formed (Figure VI), and the method must determine them as though they were identical. Finally, the method must be completely free from interference by hydroperoxides since these are present in far greater quantity than are the nitro-β-hydroperoxides.[9]

REVIEW OF LITERATURE METHODS FOR THE DETERMINATION OF THE NITRO-β-HYDROPEROXIDES

A literature search was carried out in an attempt to find a method which would meet the criteria listed above. It was ultimately determined that none of the literature methods for the determination of aliphatic nitro compounds were satisfactory. In the following brief review of those methods, the methods are categorized either according to the species which is determined in or by the major reagent used for the analytical finish.

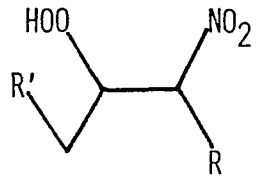
DETERMINATION AS NITRO

Redox Determinations

Many nitro determinations rely on a reductive titration.[12-18] However, these methods, as well as those using polarography or coulometry,[19-21] are unsatisfactory since they are subject to interference by hydroperoxides, and as stated earlier, the hydroperoxide concentration is much greater than the nitro-β-hydroperoxide concentration.[9] In addition, these methods are not sufficiently sensitive for a micro determination.

FIGURE VI

ISOMERIC NITRO-BETA-HYDROPEROXIDES



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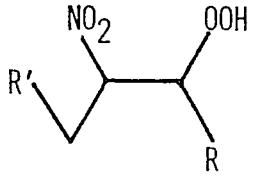

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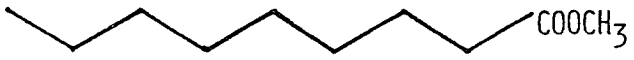
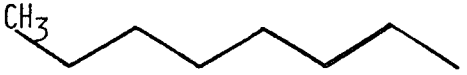
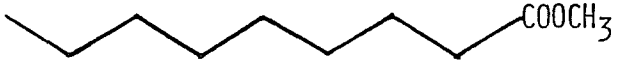
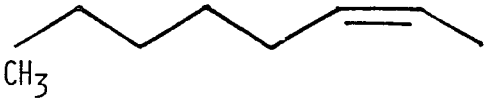
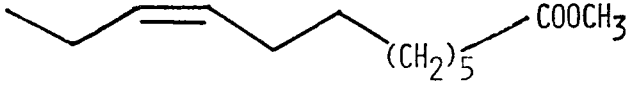
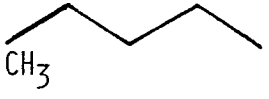
18:1 R = 

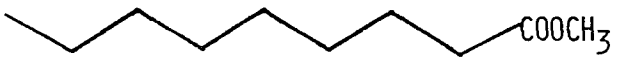
R'= 

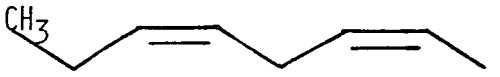
18:2 R_A= 

R'_A= 

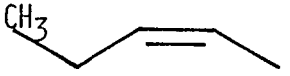
R_B= 

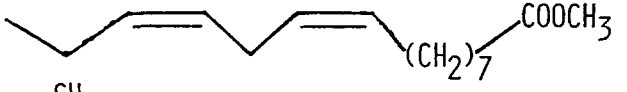
R'_B= 

18:3 R_A= 

R'_A= 

R_B= 

R'_B= 

R_C= 

R'_C= 

Total Nitrogen Determinations

Both the Kjeldahl and the Dumas total nitrogen methods are unsatisfactory for the determination of nitro-β-hydroperoxide in the autoxidation system. The Kjeldahl method is too insensitive for small samples containing low concentrations of nitro compounds.[22] Also, the method is particularly difficult for organic compounds containing N-O bonds,[23] and any adaptation which determines these refractory compounds is very time consuming.[24] The Dumas method has been shown to be unacceptable for fatty nitrogen compounds, and its sensitivity is too low for micro determinations.[23]

Nitrous Acid Methods

Turba et al.[25] and Altshuller and Cohen[26] used the reaction of nitrous acid with nitro compounds for their determination methods. In the case of secondary nitro compounds such as the nitro-β-hydroperoxide, the pseudo nitrole (Figure VII) is formed. These methods are too insensitive for micro scale determinations.

Aci Ion Methods

A large number of methods utilize the formation of the aci tautomer (Figure VIII) of the nitro group.[27-29] For secondary nitro compounds, however, the equilibrium does not favor the aci tautomer[30] unless it is stabilized, for example, by resonance as in the 2-nitro-1, 1-bis(p-chlorophenyl) alkanes.[28] Unfortunately, no compound has been found which reacts with the aci form irreversibly to force the equilibrium to completion. The Fe^{+3} complex, for example, even if formed, reverts quickly to the nitro compound.[31]

FIGURE VII

PSEUDONITROLE

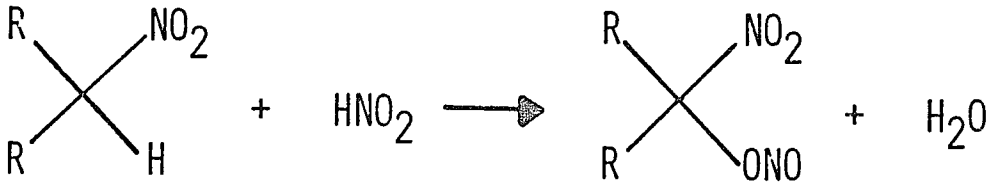
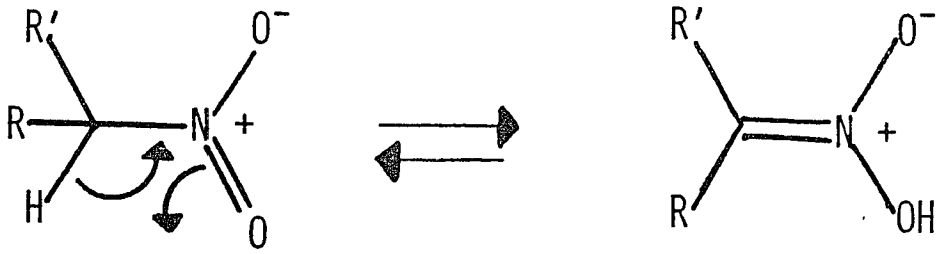


FIGURE VIII

ACI-ION TAUTOMERIZATION



Acid or Base Reagent Methods

Grebber et al. determined nitro compounds quantitatively using H_2SO_4 and diphenylamine,[32] but this test cannot be used in the presence of hydroperoxides. Ashworth stated that the Janovsky reaction, which Ashworth incorporated into a determination method for primary nitro compounds, was too erratic for use with secondary nitro compounds.[33]

Spot Test Methods

The possibility of developing a spot test into a quantitative method was investigated. However, none of the spot methods can be used for the determination of nitro-β-hydroperoxides,[34-36] either because they detect only aromatic nitro compounds or because hydroperoxides interfere with the method.[37]

DETERMINATION BY CONVERSION TO NITRITE ION

Methods Using Acid

Methods which form nitrite ion by the action of strong acid, such as those of Gwatkin[38] and Jones and Riddick,[39] are unacceptable since lipid decomposes to an intractable tar in acid sufficiently strong to oxidize the nitro compound to nitrite ion.

Methods Using Base (The Sweet Method)

The oxidation of nitro compounds to nitrite ion by treatment with hydrogen peroxide and strong base has been successfully used to quantify aliphatic nitro compounds and nitrite esters. One of these methods,

first used as a spot test by Bose,[40] and later developed into a quantitative method by Sweet et al.,[41] seemed suited to the determination of nitro-β-hydroperoxides in the autoxidation system.

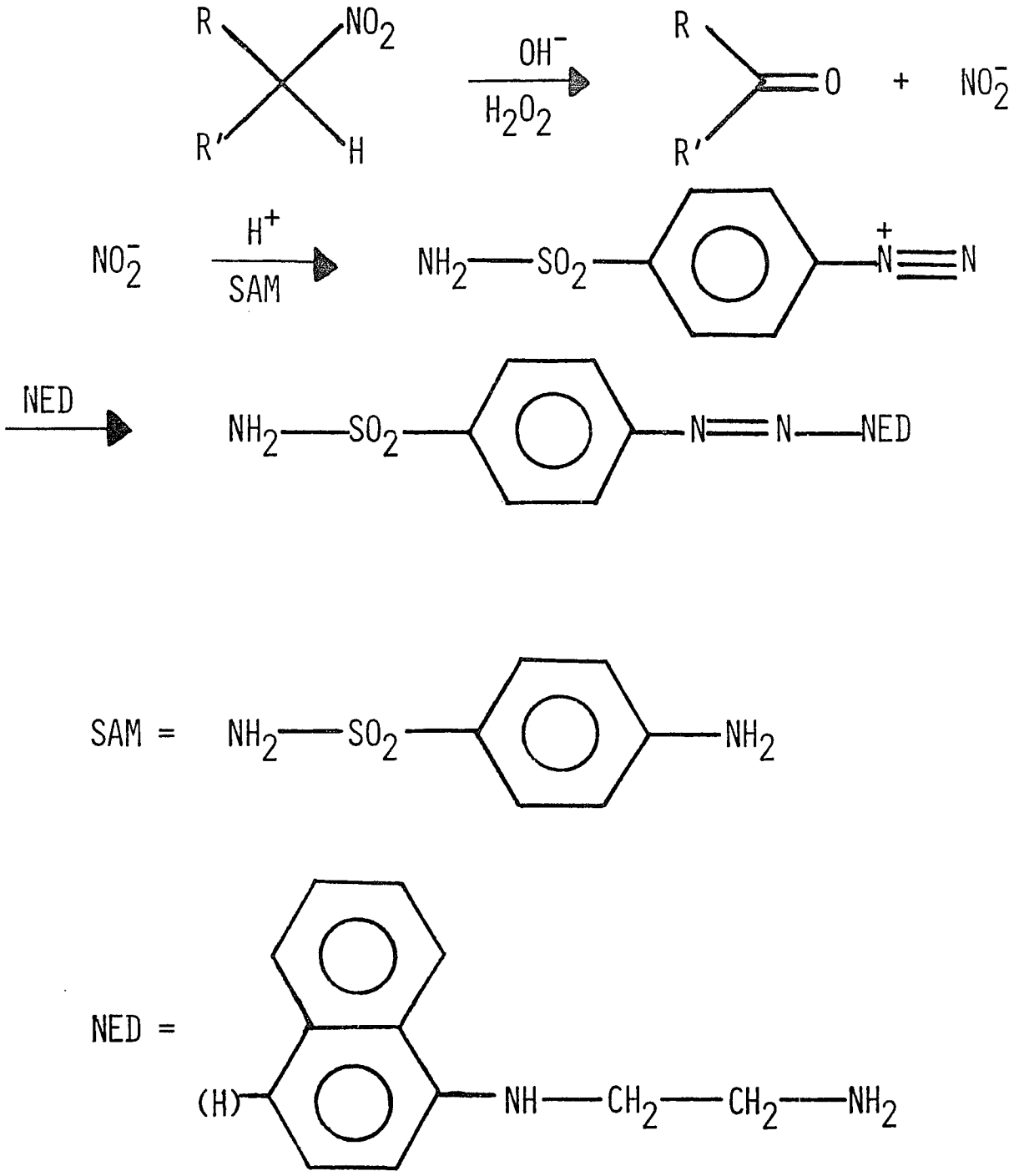
Under the stringent conditions used, the same yield of nitrite ion was expected from each of the isomeric nitro-β-hydroperoxides. Thus, the method was expected to be independent of the position of the nitro group along the carbon chain. Since concentrated hydrogen peroxide is one of the test reagents, the hydroperoxides present in the lipid were not expected to interfere. Lastly, the method uses the sensitive Griess-Ilosvay method (formation of diazotized sulfanilamide with the nitrite ion from the decomposition of the nitro compound, followed by coupling with N-1-naphthylethylene diamine dihydrochloride (Figure IX)) which provides very high sensitivity.[42,43]

Sweet found that his initial conditions gave erratic and low yields of nitrite ion from 1-nitrodecane.[41] He was able, however, to obtain an acceptable yield of nitrite ion by increasing the base concentration. It was hoped, therefore, that his conditions for 1-nitrodecane, or more stringent ones, would give an acceptable, although not a quantitative, yield of nitrite ion from nitro-β-hydroperoxides.

Unfortunately, our investigations showed that this was not the case. The method of Sweet,[41] the modifications of Meisel and Erdy[44] and Estes and Baughman,[45] and our modifications all failed to give an acceptable yield of nitrite ion (see both the Experimental and the Results and Discussion Sections).

FIGURE IX

THE SWEET DETERMINATION SEQUENCE



DEVELOPMENT OF A DETERMINATION METHOD

Since no literature method could be found by which the nitro-β-hydroperoxides formed in the autoxidation system could be satisfactorily determined, a new method was developed. The method involves the reduction of the nitro group to an amine followed by the spectrophotometric determination of the amine.

REDUCTION OF NITRO COMPOUNDS TO AMINES

Metal Catalyzed Reductions

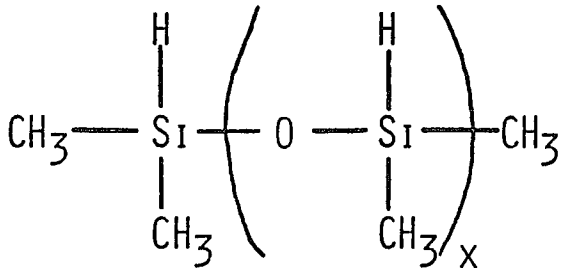
Several methods of catalytic hydrogenation[46,47] and reductions using metal ions[24,48,49] are described in the literature. These methods are time consuming, however, do not work well for aliphatic nitro compounds, and do not lend themselves well to a subsequent determination of the amine formed. Reduction with lithium aluminum hydride has been used to prepare amines from nitro compounds,[49-51] but several experimenters have found that reductions of aliphatic nitro compounds with this reagent are not satisfactory. In particular, difficulty has been found in the case of β-hydroxy nitro compounds, which would be formed during the reduction of nitro-β-hydroperoxides.

Hydrosiloxane Reductions

In 1957, Nitzsche and Wick[54] reduced several aromatic nitro compounds to the corresponding amines using PolyMethylHydroSilane (PMHS) (Figure X) and a metal alkoxide or acylate as a catalyst. More

FIGURE X

PolyMethylHydroSilane (PMHS) *



* For the Dimer, x = 1.

recently, Lipowitz and Bowman[55] used milder conditions to obtain the same reductions. Using PMHS and 5% Pd on activated charcoal, they were able to obtain aniline in 89% yield from the reduction of nitrobenzene. When the dimer, $(Me_2SiH)_2O$, was used with the same catalyst in aqueous dioxane, aniline was produced in 100% yield.[56]

Our investigations showed that the same system reproducibly reduced aliphatic nitro compounds as well (see Experimental Section). Thus, the PMHS plus Pd on charcoal reduction system is well suited to the reduction of the nitro-β-hydroperoxides. A reproducible yield of the amine can be expected from the reduction, and the homogeneous system lends itself to a subsequent spectrophotometric determination.

Hydroperoxides are reduced to alcohols by these conditions,[55] and do not interfere. Therefore, it was only necessary to find a sufficiently sensitive method for determining the alcohol-amines formed by the reduction of the nitro-β-hydroperoxide.

DETERMINATION OF ALCOHOL-AMINE

Alcohol Interference

A number of the reported methods for the determination of aliphatic amines are subject to interference from alcohols,[57-71] and these determinations were eliminated from consideration.

Imine Determination Methods

Several amine determinations are based on the formation of an imine with salicyaldehyde or one of its derivatives.[72-76] Although the imine can be formed from the nitro-β-hydroperoxide, none of these methods are sufficiently sensitive.

Spot Test Methods

A number of spot tests for amines were eliminated because they are too insensitive or because there is some species in the lipid system which interferes.[61,62,77-83] The determination of Valentine, Entrikin, and Hanson, for example, requires anhydrous conditions.[77]

Indicator Dye Methods

Of the remaining literature methods,[84-94] those which involved

the use of a pH indicator dye seemed particularly suited to the determination of the alcohol-amines formed from the reduction of the nitro-β-hydroperoxides.

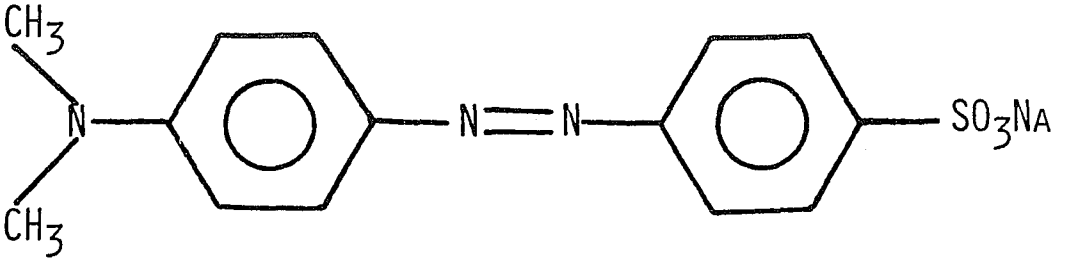
These methods rely upon the formation of a complex or a salt between the amine and the indicator dye. The complex is then determined spectrophotometrically, either in the original solution or after extraction. The dye methods meet all of the requirements of the lipid system. They are extremely sensitive, most are capable of determining a few micromoles of an amine. The alcohols which are formed from the reduction of hydroperoxides do not interfere with these methods. Since the chromophore is approximately the same for any amine, i.e., the amine salt of the indicator dye, the extinction coefficient is almost independent of the amine itself. Thus the variety of amine isomers formed from the various nitro-β-hydroperoxides would all have essentially the same extinction coefficient. Lastly, those methods which use an extraction step facilitate the formation of a clear solution for the spectrophotometric determination, and the extraction can be used to increase sensitivity.

The Methyl Orange Method

One of the dye extraction methods, developed by Silverstein,[84] uses the salt formed by methyl orange and a fatty amine for the determination of the amine. In the procedure, the sulfonic acid anion of methyl orange (Figure XI) forms a neutral salt with the protonated amine. This neutral salt is then extracted into chloroform leaving behind all unreacted dye. A measurement of the amount of amine-dye salt in the organic layer is, therefore, a measure of the amount of

FIGURE XI

METHYL ORANGE



amine in the test sample. The salt formation and extraction are forced to completion by repeated extraction steps.

This method, subsequent to the PMHS reduction to the nitro-β-hydroperoxides, proved satisfactory for the determination of the nitro-β-hydroperoxides from the autoxidation system, in addition to other long chain nitro compounds (see Experimental Section).

MATERIALS AND EQUIPMENT

Water, unless otherwise noted, was distilled and then deionized by passage through a mixed bed column.

N-1-Naphthalethylene diamine dihydrochloride, reagent grade, was obtained from J. T. Baker.

Sulfanilamide, reagent grade, was obtained from Fisher Scientific Co.

All lipids (PUFA) were obtained from Sigma Chemical and were used without further purification.

Dodecyl amine was obtained from Aldrich Chemical Co., and was used without further purification.

2-Nitropropane was obtained from Aldrich Chemical Co., and was distilled before use.

Salicylaldehyde was obtained from Matheson Coleman Bell.

Polymethylhydrosilane was obtained from Aldrich Chemical Co.

Palladium on charcoal (5%) was obtained from the Alfa Division of Ventron Chemicals.

Butylated hydroxytoluene was obtained from Aldrich Chemical Co.

1-Iodohexadecane was obtained from Eastman Chemicals.

Dinitrogen tetroxide was obtained from Matheson gases.

6-Bromohexanoic acid was obtained from Aldrich Chemical Co., and was used without further purification.

Dodecanal was obtained from Aldrich Chemical Co., and was purified immediately before use by passage through a plug of silica to remove acid.

Dicyclohexyl-18-crown-6 was obtained from Aldrich Chemical Co., and was used without purification.

IRA 400 resin was obtained from Sargent Welch Co.

Trimethyl hexadecyl ammonium bromide was obtained from Aldrich Chemical Co., and was used without further purification.

Sebacic acid was obtained from Aldrich Chemical Co., and was used without further purification.

1-Bromooctane was obtained from Aldrich Chemical Co., and was used without further purification.

Hexanoic acid was obtained from Aldrich Chemical Co., and was used without further purification.

2-Nitrooctane was obtained from ICN K and K, and was purified by vacuum distillation.

All other chemicals were reagent grade or better.

Spectrophotometers used were the Cary model 14 and the Beckman DU

(modified with a Gilford power supply, photomultiplier, and sample holder).

NMR spectra were obtained using a Varian A-60.

IR spectra were obtained using a Perkin-Elmer 621.

GLPC separations were carried out using a 6-foot DEGS column (15% on Chromosorb P) with the flame ionization detector of a Glowall 320 gas chromatograph, unless otherwise specified.

All glassware used for volumetric work was Class A.

EXPERIMENTAL PROCEDURES

NITRITE DETERMINATIONS

The Sweet Method[41]

The sample (10-30 mg) is placed in a flask containing 50 ml of water and 5 ml of 25% (50% for medium chain nitro compounds) NaOH solution. To this is added 3.5 ml of 30% hydrogen peroxide. The flask is then fitted with a reflux condenser and heated in the following manner: 30 minutes at 45-50° C, 10 minutes at 60° C, 10 minutes at 80° C, and 30 minutes at 100° C. The flask is then cooled to room temperature, and the contents are transferred to a 100 ml volumetric and diluted to the mark. An aliquot is then taken for determination To the aliquot are added 2 ml of the NED reagent and 2 ml of the SAM reagent. Absorbance is measured within 30 minutes at 520 nm.

REAGENTS:

SAM: Sulfanilamide, 35 g, is added to 250 ml of H_3PO_4 in a 1 liter volumetric and the contents are diluted to the mark.

NED: N-1-naphthylethylene diamine dihydrochloride, 25 mg, is dissolved in 100 ml of water.

The Estes and Baughman Method[45]

A 0.2-2.2 μmole sample is added to 5 ml of water to which 1 ml of a 6.3 N NaOH had been added. The container is shaken well, allowed to

stand for 10 minutes, and then 0.5 ml of 30% hydrogen peroxide is added. The container is loosely stoppered and heated for 1-1.5 hours in an 80° C water bath. Then the container is cooled, and the contents are transferred to a 10 ml volumetric and diluted with water.

A 1 ml aliquot is placed in a spectrophotometric cell, 1 ml of each of the reagent solutions is added, and the absorbance is measured between 15 and 30 minutes later at 540 nm.

REAGENTS:

SAM: Sulfanilamide, 0.2 g, is dissolved in 20 ml of 1:4 HCl.

NED: The N-1-naphthylethylene diamine dihydrochloride solution is 0.02% in water.

POLYMETHYLHYDROSILANE REDUCTION[55]

About 0.1 mole of sample is added to 40 ml of the acid solution, and 0.05 g of 5% Pd on charcoal and a 10% excess of PMHS are then added. The mixture is shaken and placed in a 40-60° C water bath for 30 minutes (during reaction, the mixture is shaken about every 5 minutes).

After reaction, the catalyst is removed by filtration through Whatman 1 filter paper, and the filtrate is collected in a separatory funnel. Water, 80 ml, is added to the funnel and the product is extracted with chloroform. After extraction, the organic layer is dried over $CaCl_2$, and the solvent is removed by rotary evaporation.

REAGENTS:

Acid Solution: Concentrated HCl, 1 drop, is dissolved in 40 ml of 95% ethanol.

METHYL ORANGE AMINE DETERMINATIONS

Original Silverstein Method[84]

A mixture of 4 ml of buffer, 2 ml of the methyl orange solution, 20 ml of chloroform, and 100 ml of an aqueous solution of the test sample are shaken together for 5 minutes. After allowing the layers to separate for 3-10 minutes, the chloroform layer is collected, and a second extraction is carried out with an additional 20 ml of chloroform. The combined chloroform layers are placed in a 50 ml volumetric and 2 ml of ethanol is added. The solution is diluted with chloroform, and the absorbance is measured at the maximum, 430 nm, during the next 2 hours.

REAGENTS:

Buffer: Potassium chloride, 125 g, and sodium acetate (anhydrous), 42.19 g, are added to a 500 ml volumetric. Then 300 ml of glacial acetic acid is added, and the flask is diluted with water. The pH is checked with a pH meter and adjusted with 1 N NaOH or acetic acid, as necessary, to produce a pH of 3.5.

Methyl Orange Solution: Methyl orange is dissolved in water to produce a 0.05% solution.

Adapted Methyl Orange Method

The sample is filtered into a separatory funnel, water, 50 ml, buffer, 4 ml, methyl orange solution, 2 ml, and a chloroform wash of the filter, 2 ml, are added to the funnel, and the amine-methyl orange salt is extracted. After 3-10 minutes, the chloroform layer is collected, and two additional 2 ml chloroform extractions are carried out. The combined extractions are placed in a 10 ml volumetric and 2 ml of ethanol is added to clarify the solution. After dilution, the absorbance is determined at 485 nm vs a chloroform blank.

LONG CHAIN ALIPHATIC NITRO COMPOUND DETERMINATION METHOD

The sample is placed in a test tube containing 5 ml of the ethanol/HCl reagent, and a spatula tip (ca. 0.025 g) of Pd on charcoal (5%) and 0.3 ml of PMHS are added. The mixture is swirled and placed in a water bath (40-60°C) for 30 minutes. After reaction, the catalyst is removed by filtration and the liquid is collected in a separatory funnel. A 2 ml chloroform wash of the test tube is also filtered into the funnel. Water, 50 ml, buffer, 4 ml, and methyl orange solution, 2 ml, are added to the funnel and the amine complex is extracted into the chloroform layer. After allowing the layers to separate for 3 minutes, the chloroform layer is collected in a 10 ml volumetric, and two additional 2 ml chloroform extractions are then collected in the same manner. Ethanol, 2 ml, is added to the volumetric to clarify the solution, and the flask is diluted to the

mark with chloroform. At any time during the next 2 hours, the absorbance at 485 nm is measured vs a chloroform blank. Care must be taken to prevent the inclusion of any of the aqueous layer, in order that the methyl orange it contains does not interfere.

SYNTHESIS OF PRIMARY NITRO COMPOUNDS

Reaction of Primary Halides with Sodium Nitrite[95]

1-Iodohexadecane, 53 g, is mixed with 18 g of $NaNO_2$ in about 300 ml of DMSO, and the mixture is stirred at room temperature for 5 hours. At the end of this time the mixture solidifies. Addition of chloroform produces a partial solution, and addition of water dissolves the remaining material into a second layer. The two layers are placed in a separatory funnel, shaken, and allowed to separate. The chloroform layer is extracted five more times with water to remove NaI and DMSO, and is dried over anhydrous $MgSO_4$. After filtration, the chloroform is removed by rotary evaporation. The product (about 70% nitro compound, 25% nitrite ester, and 5% unreacted halide) is separated by precipitation of the nitro carbanion salt. Sodium, 2.5 g, is added to 20 ml of methanol, and this solution is added to a solution of the product mixture in 40 ml of methanol. The salt of the anion of the nitro compound forms immediately, and is collected by filtration. The precipitate is washed extensively with diethyl ether to remove nitrite ester and halide, and is partially dried by pulling air through the filter. The salt is then added to a separatory funnel containing chloroform and aqueous hydrochloric acid. After mixing,

additional acid is added until the solution remains at a pH of about 2. The chloroform layer is then collected and dried over $MgSO_4$. The chloroform is removed by rotary evaporation, producing a clear and colorless oil.

An NMR spectrum of the material showed that it was free of both nitrite ester and unreacted halide. This material was used, without further purification, to standardize the nitro determination.

ATTEMPTED SYNTHESES OF SECONDARY NITRO ESTER

Methyl Oleate + HBr

HBr gas is bubbled slowly from a lecture bottle into a solution of 10 g of 18:1 in 200 ml of CCl_4. After 30 minutes, the addition is stopped. Thereafter, HBr is added for 5 minutes every two hours to maintain color in the solution. After 20 hours, reaction is stopped, the HBr is allowed to escape, and the solvent is removed by rotary evaporation. The oil is then chromatographed on silica using 5% diethyl ether and n-hexane. The eluant is collected and reduced by rotary evaporation. The material is then chromatographed using silica gel and pentane (stirred over concentrated H_2SO_4, and distilled).

An NMR spectrum of the clear, colorless oil showed no impurities, and gave the correct 1:3 ratio of methine to methyl ester protons.

Methyl 9 or 10-Bromooctadecanoate + Sodium Nitrite[95]

Methyl 9 or 10-bromooctadecanoate, 5 g, is added to a flask containing 10 ml of DMSO and 1.56 g of $NaNO_2$, and the mixture is stirred

at room temperature for 5 hours. The mixture is poured over 25 g of ice layered with 5 ml of petroleum ether, the layers are separated in a separatory funnel, and the aqueous layer is extracted four more times with petroleum ether. The combined ether layers are then extracted five more times with water, dried over anhydrous $MgSO_4$, filtered, and the solvent is removed by rotary evaporation.

Identification and Separation Attempts

Although the NMR spectrum of the product mixture did not indicate that any nitro compound had been formed, it was possible that the nitro compound might have been produced in low yield. Therefore, a separation was attempted. Ethyl acetate:cyclohexane 1:2, 1:4, 1:1, 2:1, and 4:1 and the same ratios of chloroform:cyclohexane were used in attempts to isolate the nitro compound. None of the spots isolated gave the correct NMR quintet for the methine proton.

Crown Ether Catalysis[96]

The substitution reaction is repeated using a crown ether catalyst. A halide:$NaNO_2$:dicyclohexyl-18-crown-6 mole ratio of 20:22:1 is used.

An NMR spectrum indicated that no methyl 9 or 10 nitro octadecanoate had been formed.

Tetralkyl Ammonium Catalysis[97]

The substitution reaction is repeated using hexadecyl trimethyl ammonium bromide as a catalyst (0.5%).

Again an NMR spectrum showed that no reaction had occurred.

ATTEMPTED SYNTHESIS OF SECONDARY NITRO COMPOUND

Synthesis of n-Butyl Tosylate[98]

A 500 ml round bottom is placed in a cooling bath held at 12°C, and a thermometer is placed in the flask to monitor temperature. After precooling the flask to 12°C, 10 g of n-butanol and 15.25 g of p-toluene sulfonyl chloride are added to the flask. NaOH, 23 ml of 5 N, is added dropwise over a 2 hour period, while maintaining the temperature below 15°C. Then a second portion of p-toluenesulfonyl chloride is added, and another 23 ml of 5 N NaOH is added dropwise. After completion of the addition, the mixture is stirred for an additional 4 hours. The mixture is then partitioned between 10% NaOH and hexane, the hexane layer is extracted four times with 10% base, and the hexane layer is dried over anhydrous $NaCO_3$, filtered, and is reduced by evaporation. n-Butyltosylate is isolated in 98% yield.

Reaction of n-Butyltosylate with 1-Nitrooctane

The tosyl chloride is reacted with 1-nitrooctane according to the procedure for hexanoyl chloride and nitromethane.

Only unreacted starting materials were recovered from the reaction.

ATTEMPTED SYNTHESES OF NITRO-β-HYDROPEROXIDE ESTER

Methyl Oleate + NO_2 (ppm) in Air

Methyl oleate, 2 g, is mixed with an equimolar portion of BHT

and the mixture is exposed to 10 ppm of NO_2 (delivered from an impinger tube)[9] in air for 10 hours.

A thin layer chromatographic separation of the product mixture was attempted. No spot could be found which gave the correct NMR spectrum. Solvents used were 4:1, 2:1, 1:1, 1:2, and 1:4 ethyl acetate: cyclohexane and the same ratios of ethyl acetate:chloroform.

Methyl Oleate + N_2O_4 in Oxygen Atmosphere[99]

Dinitrogen Tetroxide (N_2O_4) is condensed from a lecture bottle into a calibrated ice trap. A known amount, 4.14 ml, is then transferred in a stream of oxygen to a round-bottom flask equipped with a mechanical stirrer, a dry ice condenser, a constant pressure addition funnel, and a gas inlet and outlet. The flask, containing 33 ml of diethyl ether, is kept at 9-11°C throughout the reaction. After addition of the N_2O_4, 32 ml of 18:1 is added through the addition funnel over a 45 minute period. A slow stream of oxygen is blown through the reactor during the addition. After the addition is completed, the flask is allowed to warm to room temperature, and is then stirred for an additional 35 minutes. Diethyl ether, 270 ml, and an excess of 2 N H_2SO_4 are added, and the mixture is stirred overnight. The organic layer is then collected and the aqueous layer is extracted twice with ether. The combined ether fractions are reduced by rotary evaporation, after drying over anhydrous $MgSO_4$.

Separation Attempts

Separation by GLPC and TLC (the latter using hexane:isopropanol: acetic acid 96:2.6:014) showed four or perhaps five compounds in

addition to unreacted starting material. At least one of these gave a multiplet at $\delta = 4.6$ as predicted for the methine proton alpha to the nitro group.[100] Several TLC solvent systems were tested in an attempt to isolate the nitro-β-hydroperoxide. Hexane:isopropanol: acetic acid 96:2.6:0.4 did not give complete separation. Diethyl ether:petroleum ether:methanol mixtures of 0:0:1, 1:1:0, 2:1:0, 2:1:1, 2:1:2, and 4:1:5 were tried, but none gave pure nitro-β-hydroperoxide. Finally, separation was attempted using porapak normal phase HPLC and C_{18} reverse phase HPLC. However, neither method proved satisfactory. Since the yield of NBH was so low from this reaction, and since no means could be found which would separate the product mixture, the synthetic method was abandoned.

ATTEMPTED SYNTHESES OF NITRO-β-ALCOHOL

Esterification of 6-Bromohexanoic Acid

The acid, 10 g, is dissolved in 20 ml of methanol containing 3.6 ml of concentrated H_2SO_4, is refluxed for 4 hours, cooled, and added to a separatory funnel containing 12 ml of water and 10 ml of diethyl ether. The ether layer is separated and then washed with 5 ml portions of water, 5% $NaCO_3$, and saturated NaCl. The organic layer is dried over anhydrous $MgSO_4$, filtered, and reduced by rotary evaporation. Pure methyl 6-bromohexanoate is obtained in a 95% yield.

Methyl 6-Nitrohexanoate + Dodecanal (IRA 400 Resin Catalysis)[101]

IRA 400 ion exchange resin is pretreated by several washings with

5% NaOH followed by several washings with 95% ethanol. Methyl 6-nitrohexanoate, 3 ml, is added to a 20% excess of dodecanal, 3 g, in 20 ml of ethanol. The resin, 0.76 g (25% of aldehyde by weight), is added, and the mixture is stirred at room temperature for 24 hours.

No reaction occurred at room temperature, and when the reaction was repeated in refluxing 95% ethanol, an NMR spectrum showed that only self-condensation of dodecanal had occurred.

Methyl 6-Nitrohexanoate + Dodecanal (Sodium Methoxide Catalysis)[101,102]

The coupling experiment described above is repeated, substituting 0.7 g of $NaOCH_3$ for the ion exchange catalyst.

Identical results were obtained.

Dodecanal + Nitromethane

The experiment described above is repeated with 0.78 g of nitromethane substituted for the methyl 6-nitrohexanoate.

When carried out at room temperature, the addition product was formed in about 90% yield, as determined by NMR integration.

Dodecanal + 1-Nitrooctane

The experiment described above is repeated with the substitution of 2.04 g of 1-nitrooctane for the nitromethane.

When the reaction was carried out at room temperature for 4 hours, NMR indicated that no reaction had occurred. When the methanol was heated to reflux for 1 hour, only self-condensation product from dodecanal was found.

ATTEMPTED SYNTHESES OF NITRO-β-KETONE

Methyl Sebacoate + 1-Nitrooctane

Methyl sebacoate is prepared from the diacid by the procedure used for the esterification of 6-bromohexanoic acid.

Sodium metal, 0.23 g, is reacted with 50 ml of methanol, and after disappearance of the metal, 1.6 g of nitrooctane is added. A slightly yellow solution is formed. NMR clearly indicates the loss of one of the alpha protons and formation of the anion of the nitro compound. The 50 ml of solution containing the carbanion is added dropwise to 150 ml of a stirred methanol solution of 5.6 g of the diester. Stirring is continued at room temperature for 4 additional hours. The solution is placed in a separatory funnel and the base is neutralized by addition of aqueous NH_4Cl. The solution is then extracted with chloroform, and the chloroform layer in turn is extracted with three portions of water. The organic layer is dried over $MgSO_4$ and reduced by rotary evaporation.

Analysis by NMR showed that no methyl 11-nitro-10-oxyoctadecanoate had been formed.

Hexanoyl Chloride + Nitromethane

Hexanoyl chloride is prepared by reaction of 20 g of hexanoic acid with an excess of thionyl chloride. The acid chloride is collected by distillation.

An apparatus consisting of a three neck round bottom flask equipped with a constant pressure addition funnel (equipped with a gas inlet) in one neck, a septum in the second, and a gas outlet in the third is

assembled hot from the oven. Argon gas is passed through the vessel continuously during the reaction. THF, 200 ml (predried over and distilled from lithium aluminum hydride), is added to the flask through the addition funnel. Then, 70 ml of 0.9 N n-butyl lithium is added by syringe through the septum. Nitromethane, 3.8 g (distilled before use), is added to 50 ml of THF, placed in the addition funnel, and added dropwise to the stirred solution. Then, 50 ml of the THF containing 8.5 g of hexanoyl chloride is added dropwise through the addition funnel. After 4 hours, the mixture is neutralized by extraction with NH_4Cl solution and the organic layer is washed four times with water, dried over $MgSO_4$, and reduced by rotary evaporation.

An NMR spectrum showed only unreacted nitromethane and hexanoic acid, which had been formed during the work-up procedure.

RESULTS AND DISCUSSION

ATTEMPTED DETERMINATION OF NITRO-β-HYDROPEROXIDE BY CONVERSION TO NITRITE ION

Discovery of Error in the Literature

Since the paper by R. L. Sweet et al.[41] is in an uncommon journal not available at LSU, preliminary studies were carried out following a description of the Sweet procedure contained in a book.[103] Unfortunately, the book contained an error; it reported that Sweet had used 2.5% NaOH as a reagent, rather than the correct 25%. Much time was lost in attempts to obtain the correct results for simple model compounds (see below) using this incorrect procedure.

Later, a paper by Estes and Baughman,[45] an adaptation of the Sweet method, was found. The authors had used a smaller sample and a simpler heating schedule. The authors also used 25% NaOH, which was an indication that the book mentioned above was in error. When the original paper by Sweet arrived, the error was proven, and all work was begun anew.

Since the adaptation of Estes and Baughman was easier and used smaller samples, it was used in all further work.

Model Compounds

Several model compounds were used to test variations of the

nitrite method, since a pure nitro-β-hydroperoxide was not available. Each variation was standardized with $NaNO_2$, and the yields reported for other compounds are calculated as the ratio of the extinction coefficient for that compound to that for $NaNO_2$. Two model nitro compounds were used for all work on the nitrite method. 2-Nitropropane was used in early studies because it was readily available. Later, 2-nitrooctane was obtained, and all further work was done with it. Although a poor model for the nitro-β-hydroperoxide, 2-nitrooctane was used in these studies because it was the best commercially available model compound. As described below, the standardization of the method for use in the PUFA system was to have been carried out via synthesis of a standard nitro-lipid. When this proved unfeasible, standardization was carried out for the PUFA system by comparing the nitro-lipid method to a total nitrogen method, using samples containing high concentrations of nitro-β-hydroperoxide.

Lipid and Hydroperoxide Interference Studies

The effects of lipid and hydroperoxide on each of the variations was measured by adding 10-500 µl of 18:2 which had been air autoxidized to about 10% reaction (as measured by the amount of hydroperoxide) to samples of the nitro compound, and then carrying out the determination. In no case did the addition of the mixture interfere with color development. However, when more than 50 µl of lipid was added, the acidification step produced a two-phase solution, and the micelles of lipid scattered the incident light in the spectrophotometric determination and gave rise to high blanks. As long as the lipid sample added was less than 50 µl, two phases did not form, and the absorbances were only

slightly higher due to scattering. This was accounted for by subtracting a blank from each sample.

The Original Estes and Baughman Procedure[45]

In initial experiments, the yield of nitrite ion from 2-nitropropane was found to be 80%. However, the yield from 2-nitrooctane was only 0.2%. Adaptation of the method was initiated in hopes of increasing the yield.

Solvent Studies

Sweet[41] had suggested that the addition of ethanol might improve the yield of nitrite ion from longer chain nitro compounds. Therefore, ethanol, acetone, and 1,4-dioxane were each tested as an added solvent to promote reaction between the very polar oxidant and the nitro compound.

Addition of ethanol, however, actually reduced the yield of nitrite ion from both 2-nitropropane and 2-nitrooctane, and 1,4-dioxane produced highly colored blanks. The addition of acetone (0.5 ml per 5 ml of solution) did increase the yield slightly, and also increased to 100 µl the amount of lipid which could be added without affecting the blank. However, the yield of nitrite ion from 2-nitrooctane, although higher, was still less than 1%. Since the addition of acetone allowed a greater amount of lipid to be taken for analysis and since the addition of acetone improved the yield of nitrite ion somewhat, all further work was carried out with the addition of 0.5 ml of acetone per 5 ml of test solution.

Increased Reagent Concentration Studies

Sweet[41] found that an increased yield of nitrite ion could be obtained from 1-nitrodecane by doubling the base concentration of the final solution (from 2.5% to 5.0%, by weight). A study of the effects of varied base and hydrogen peroxide concentrations was, therefore, carried out.

The base concentration in the final test solution was varied from its original value of 2.5% up to 50%. As the amount of base was increased, the concentration of the acid added in the neutralization step was increased a corresponding amount. The total volume, therefore, remained unchanged.

Additional base did increase the yield of nitrite ion, and the best results were obtained with 25% NaOH (Table I).

TABLE I

Base Concentration, (% NaOH)	Yield, (% Theoretical)
2.5	0.2
5.0	0.5
10.0	0.9
15.0	2.5
20.0	3.2
25.0	4.5
30.0	4.3
40.0	4.1
50.0	3.7

An investigation of the effect of increased hydrogen peroxide concentration was carried out next. The base concentration was kept at 25% and 0.5 ml of acetone was added to each sample.

The best yield of nitrite ion was obtained with a four-fold increase in the hydrogen peroxide concentration (Table II). Unfortunately, the optimum concentration gave only a 14% yield of nitrite ion from 2-nitrooctane.

TABLE II

Peroxide concentration, (% H_2O_2)	Time of Reflux, (Minutes)	Yield, (% Theoretical)
2.5	30	4.5
5.0	30	7.8
7.5	60	9.3
10.0	90	14.1
15.0	120	14.0
20.0	120	14.0

As can be seen from the table (Table II), an increase in the hydrogen peroxide concentration required an increase in the time of reflux. If any unreacted peroxide remained when the acidification and the addition of the diazotizing reagents were carried out, a reduced absorbance was observed.

Since the time of reflux increased with increased hydrogen peroxide concentration and since the yield of nitrite ion stabilized at 10.0% added peroxide, the method was considered optimized with the addition of 0.5 ml of acetone and with final concentrations of base

and hydrogen peroxide of 25 and 10%, respectively.

Determination of 2-Nitrooctane with the Adapted Procedure

When a series of samples of 2-nitrooctane were determined using the adapted method, a linear Beer-Lambert plot was obtained, although there was a significant amount of scatter (Table III).

TABLE III

2-Nitrooctane, (μmol)	Yield, (% Theoretical)
0.50	15.5
0.75	14.9
1.00	12.3
1.24	17.4
1.50	18.5
1.76	19.2
2.02	13.4
2.54	14.6
2.51	14.2

Determination of Nitro-β-hydroperoxide by the Adapted Method

Unfortunately, when the procedure was used to measure samples of 18.2 known to contain about 2 μmol of NBH, the yield of nitrite ion was less than 0.1%. Several different samples of lipid known to contain between 10 and 50 μmol of NBH were tested, but none produced sufficient nitrite ion to be detected by the spectrophotometric method.

Conclusions

It was suspected that the difference in the polarities of the reactants was responsible for the failure of the method. Some consideration was given to seeking a phase transfer catalyst which would facilitate reaction. However, the extremely low yield made the method so unattractive that further work on the method was abandoned.

POLYMETHYLHYDROSILANE REDUCTION STUDIES

Although Lipowitz and Bowman[55] had only investigated the reduction of aromatic nitro compounds by the PMHS/Pd on charcoal system, G. Grady [104] speculated that the same system would be satisfactory for aliphatic nitro compounds as well.

A sample of nitrocyclohexane was, therefore, subjected to the PMHS reduction method. Small samples were removed at intervals to follow the extent of the reaction, which was determined using GLPC with a 20% carbowax column (6 feet) held at 121°C. Reaction proceeded to completion within 30 minutes (Figure XII).

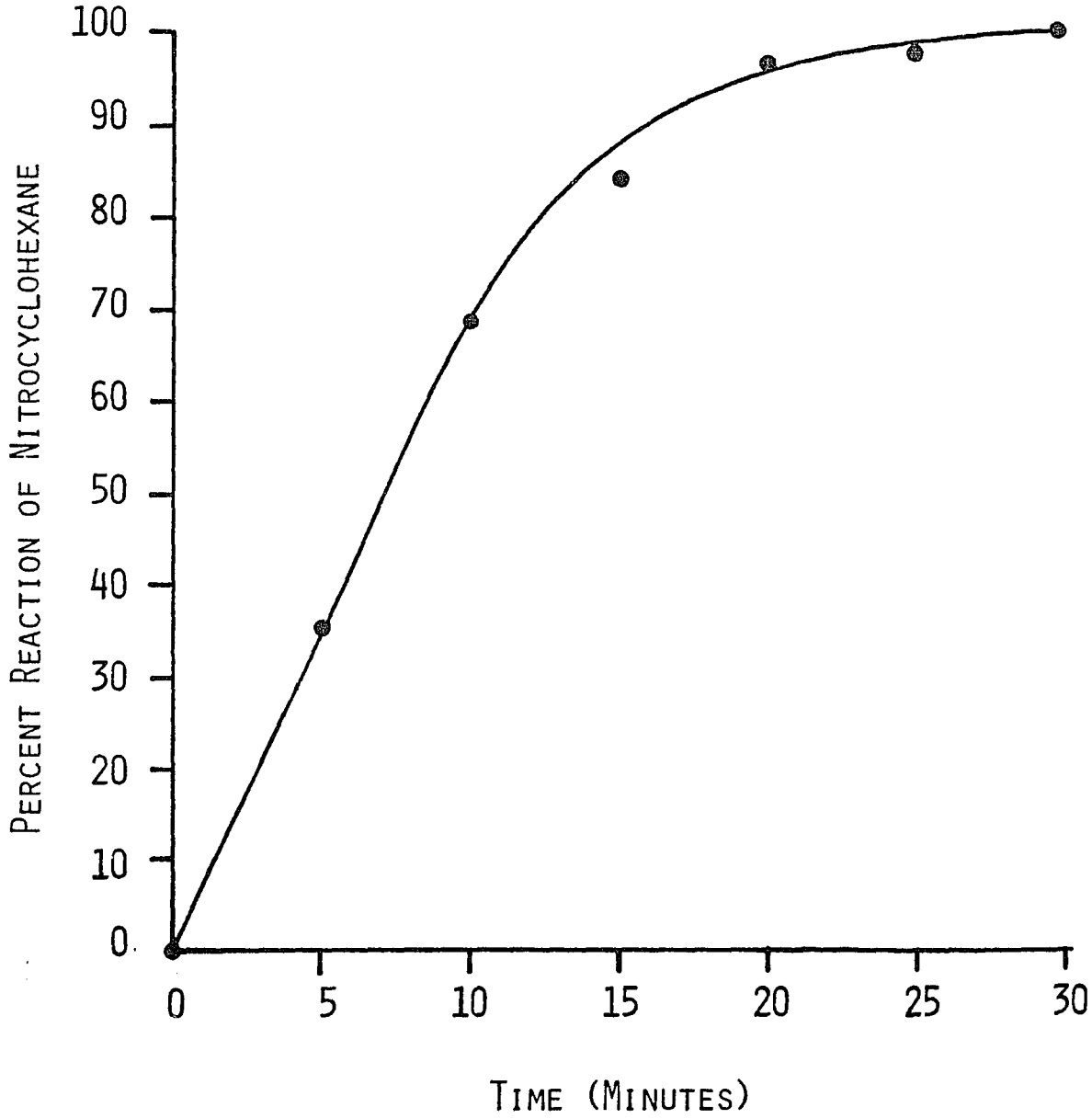
Since the yield of amine from the PMHS reduction of nitrocyclohexane was very high (quantitative within experimental error), the PMHS reduction method was incorporated into the nitro determination method without change.

METHYL ORANGE AMINE DETERMINATION

The original amine determination method developed by Silverstein[84]

FIGURE XII

PERCENT REACTION OF NITROCYCLOHEXANE VS TIME



was adapted slightly for use subsequent to the PMHS reduction method and for smaller samples (see Experimental Section). The adapted method was then tested on various amines and nitro compounds.

Determination of Dodecyl Amine by the Adapted Methyl Orange Method

Aliquots of reagent grade dodecyl amine (98%) were determined by the adapted methyl orange method. A linear Beer-Lambert plot was obtained, and the extinction coefficient* was found to be 11,520 M^{-1}-cm^{-1} (Figure XIII).

This value is slightly higher than the 9,637 M^{-1}-cm^{-1} obtained by Silverstein; the difference probably is due to the lesser purity of the industrial grade amine that he used. As can be seen from the figure, there is a positive deviation from linearity at high concentrations. This positive deviation was also found by Silverstein for all of the amines he investigated.[84]

THE NITRO LIPID DETERMINATION METHOD

Determination of Dodecyl Amine by the Nitro Lipid Procedure

When aliquots of dodecyl amine were determined by the nitro procedure (the PMHS reduction followed by the adapted methyl orange determination) an extinction coefficient of 11,440 M^{-1}-cm^{-1} was obtained (Figure XIV). Within experimental error, this was the same as the

*All extinction coefficients were determined by the linear regression method, intercepts and correlation coefficients are given with each plot.

FIGURE XIII

Absorbance (485 nm) Vs Concentration of DDA

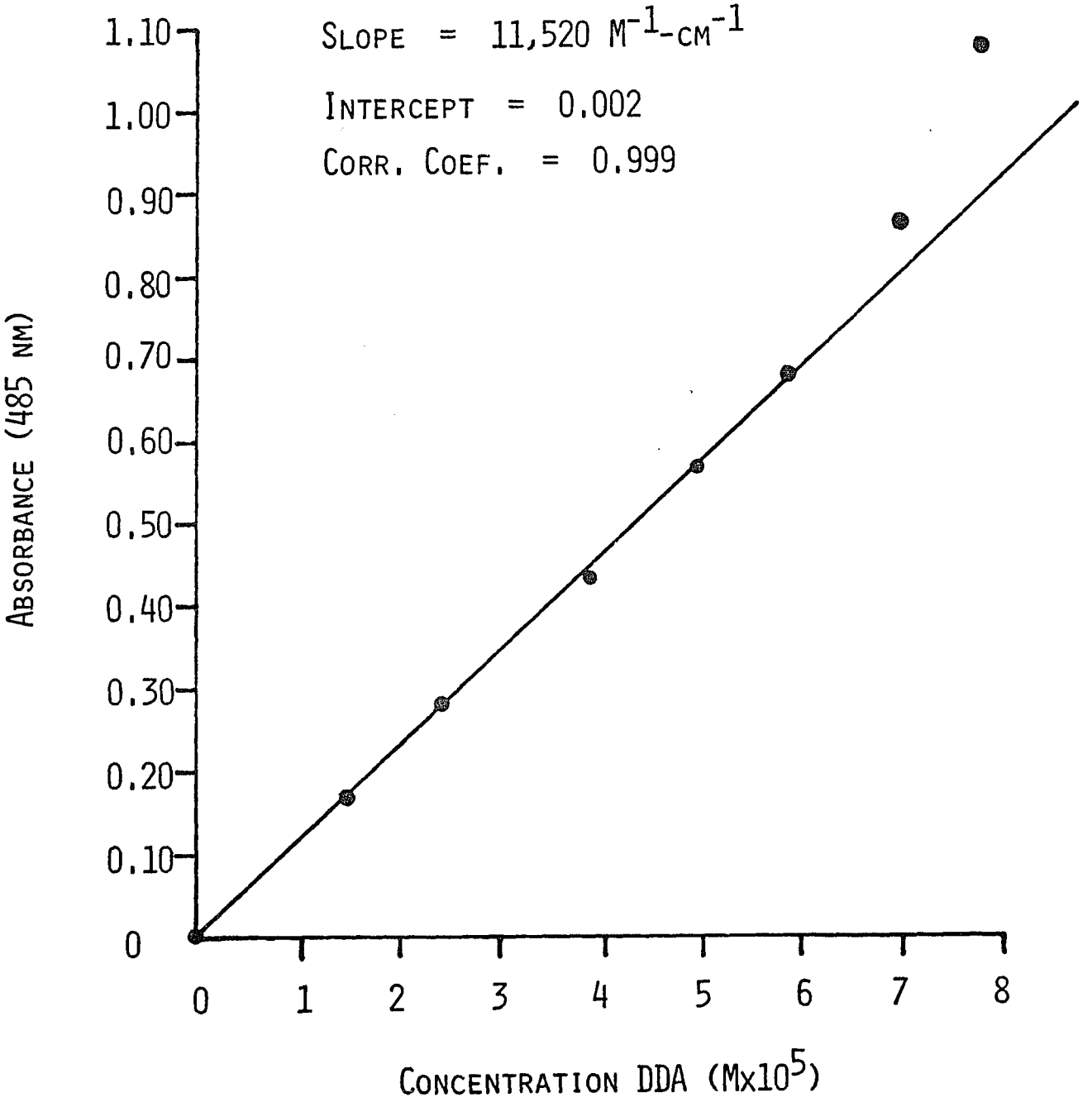
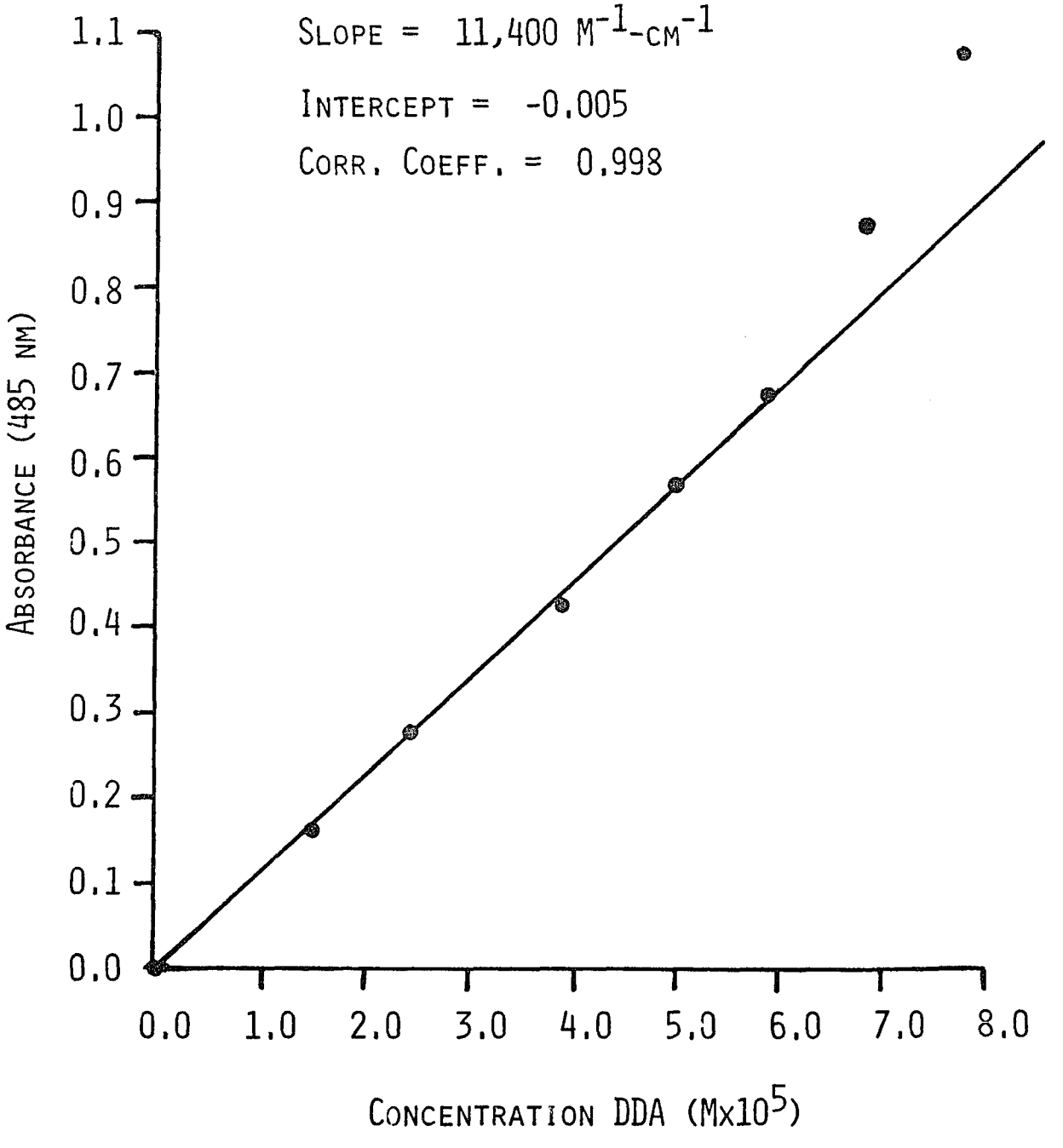


FIGURE XIV

ABSORBANCE (485 NM) VS CONCENTRATION OF DODECYLAMINE



value found for the adapted methyl orange method alone. Hence, the PMHS reduction was found not to interfere with the adapted methyl orange procedure.

Determination of 1-Nitrohexadecane by the Nitro Lipid Procedure

Aliquots of 1-nitrohexadecane in ethanol were determined by the nitro lipid method, and a linear plot was obtained (Figure XV) which gave an extinction coefficient of 6,340 M^{-1}-cm^{-1}. Although this was only 37.4% of the value that Silverstein had obtained for hexadecyl amine, the yield was consistent over a wide range of concentration. Deviation only occurred at high concentrations as had been found previously.

Determination of Nitro-β-hydroperoxide by the Nitro Lipid Procedure

When aliquots of a sample of 18:2 exposed to 2 ppm (μg/g) NO_2 were determined by the nitro lipid procedure, a linear plot was obtained over a four-fold range of concentration (Figure XVI), and the method was proven satisfactory for the determination of nitro-β-hydroperoxides in the PUFA systems

The method is currently being used to investigate several PUFA systems.[9]

Interferences

Two species are known to interfere with the method: metals which complex amines and nitrogen containing compounds which are reduced to amines by the PMHS reduction method. If these species are absent, the method provides an accurate measure of the amount of long chain nitro

FIGURE XV

Absorbance (485 nm) Vs Concentration of 1-Nitrohexadecane

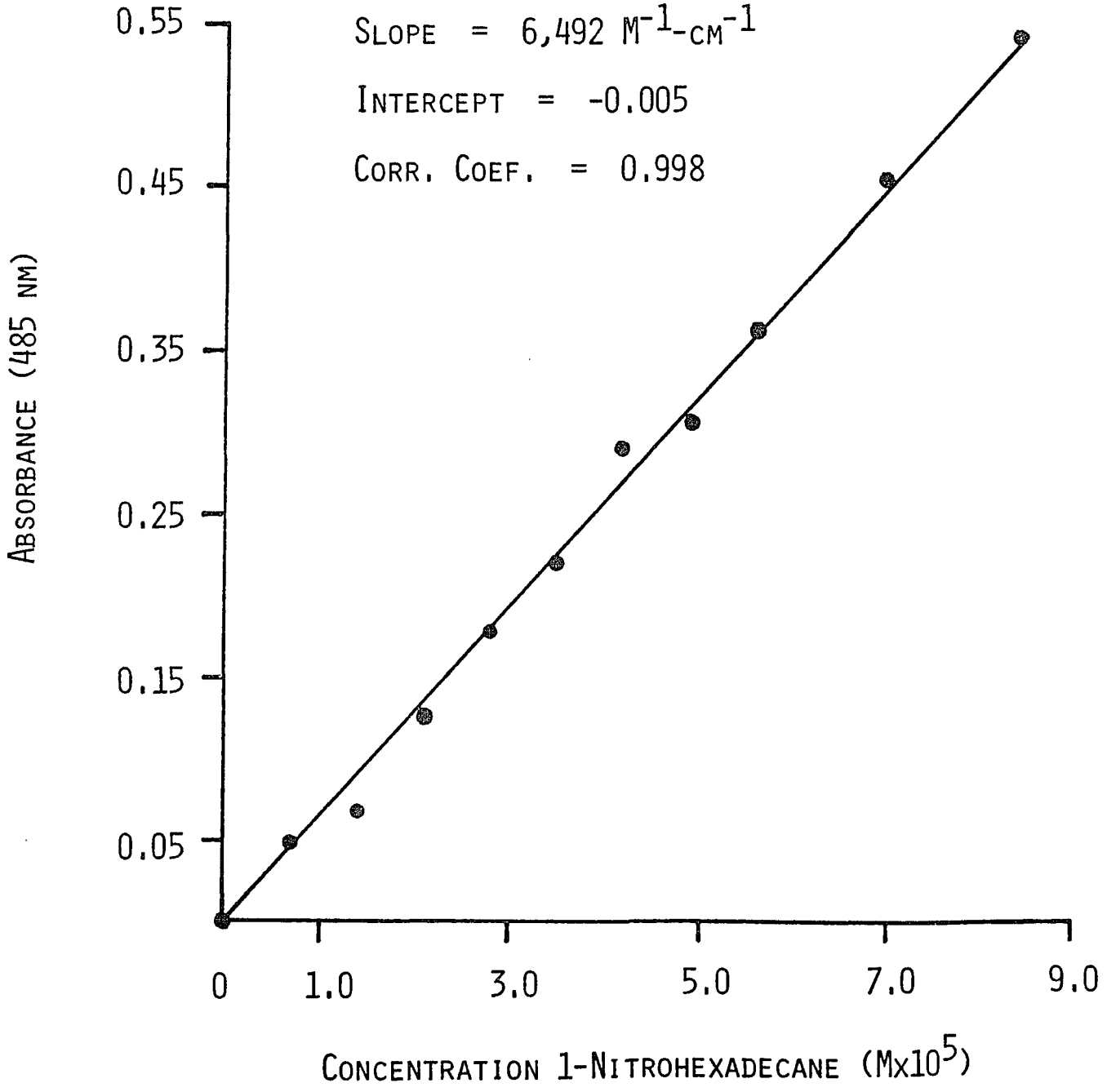
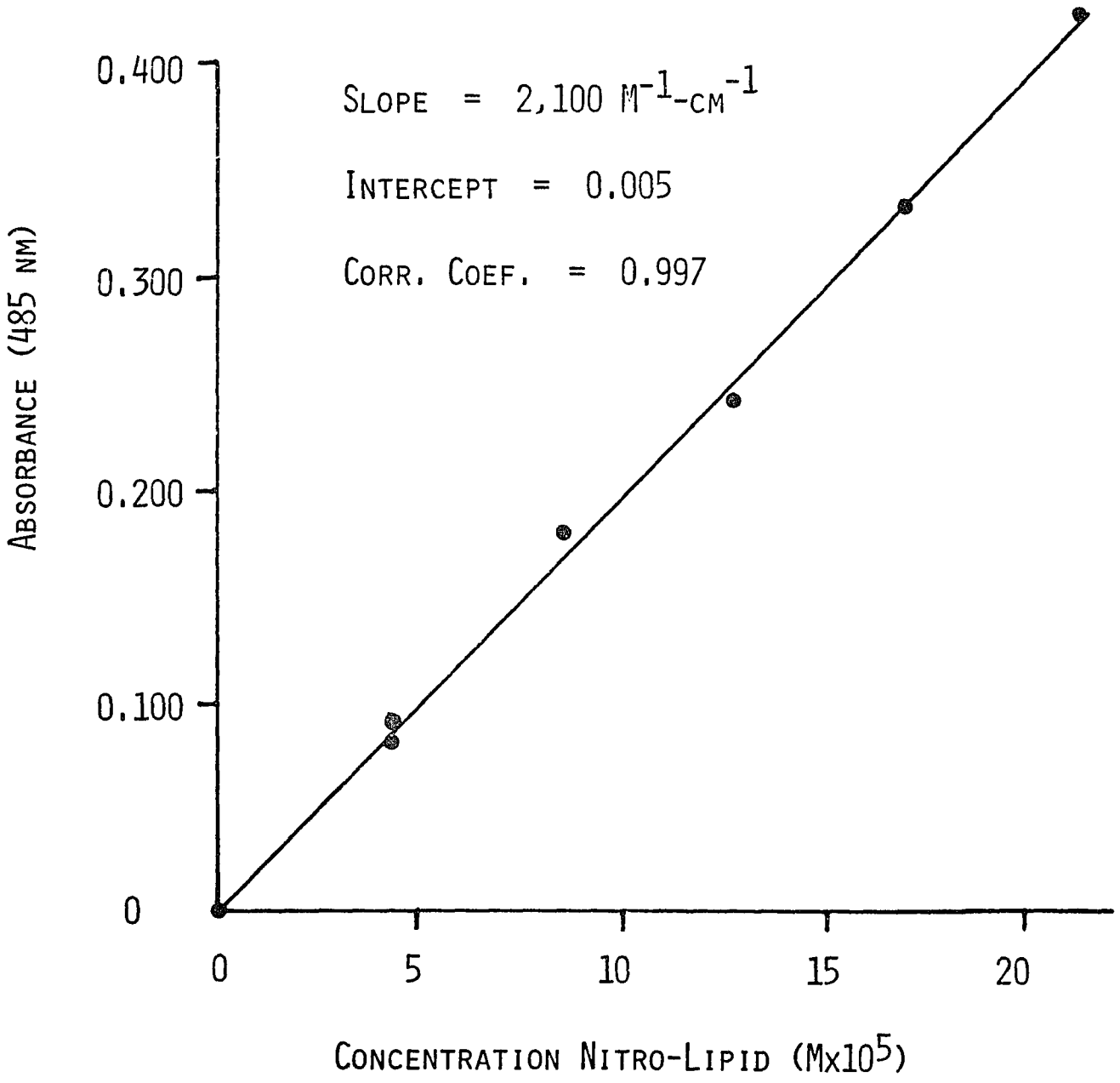


FIGURE XVI

ABSORBANCE (485 NM) VS CONCENTRATION OF NITRO-LIPID



compound present in any sample.

Determination of Other Long Chain Nitro Compounds

Any nonpolar, aliphatic nitro compound can be determined by the nitro lipid procedure, provided the amine-methyl orange complex is sufficiently nonpolar to be extracted into the chloroform layer for determination. Also, since the methyl orange method does not give the same extinction coefficient for every amine, it is necessary to standardize the method for the particular nitro compound which is to be determined, and to prepare a standardization curve in order to determine the point at which deviation from linearity occurs.

Determination of Short Chain and Polar Nitro Compounds

As stated, the method is limited to the determination of long chain, nonpolar nitro compounds by the requirement that the amine-methyl orange salt be extractable into the organic (chloroform) layer during the methyl orange determination. The method can be adapted for the determination of other nitro compounds, however, simply by using a different amine determination. One of the most general and sensitive methods for the determination of polar and short chain amines is the fluorescence method of Underfriend et al.[87] The method can be used to determine a variety of polar and short chain amines at the picomole level.

SYNTHESES OF LONG CHAIN NITRO COMPOUNDS

Synthesis of a Primary Nitro Compound by S_N2 Displacement of a Primary Halide

Since no long chain nitro compounds were commercially available, it was necessary to synthesize a long chain nitro compound to test the nitro determination. The syntheses of several nitro compounds have been carried out via the S_N2 displacement of a halide with nitrite ion,[95,105-107] and this method was used to synthesize the 1-nitrohexadecane that was used to test the nitro procedure (See above).[95]

Attempted Synthesis of a Secondary Nitro Compound by S_N2 Displacement of a Secondary Halide with Nitrite Ion

However, when methyl 9 or 10-bromohexadecanoate was reacted with sodium nitrite according to the Kornblum procedure,[95] no nitro compound was formed. Only small amounts of unreacted halide and nitrite ester were found in the product mixture.

Attempted Synthesis of a Secondary Nitro Compound via the S_N2 Displacement of a Tosylate by an α-Nitro Carbanion

Since the substitution reaction above failed to produce the desired secondary nitro compound, the synthesis was attempted via a displacement of tosylate with an α-nitro carbanion. n-Butyl tosylate was reacted with the lithium salt of the α-nitro carbanion of 1-nitrooctane (formed by the reaction of n-butyl Li with nitrooctane). Only unreacting starting materials were isolated from the reaction, and the attempted synthesis of a secondary nitro compound was abandoned.

ATTEMPTED MODEL COMPOUND SYNTHESES FOR THE STANDARDIZATION OF THE NITRO LIPID METHOD FOR THE DETERMINATION OF NITRO-β-HYDROPEROXIDES

The standardization of the nitro lipid method for the determination of the nitro-β-hydroperoxide concentration in PUFA systems was to have been carried out by the synthesis of a standard of known purity. It was hoped that either a nitro-β-hydroperoxide ester or a nitro-β-alcohol could be synthesized for use as a standard. The nitro-β-hydroperoxide ester would, of course, have been identical to the compounds from the autoxidation system. The nitro-β-alcohol would have behaved identically in the determination, since the nitro-β-hydroperoxide is reduced to the β-alcohol amine by the PMHS reduction procedure.

Several means of synthesizing both of these compounds were attempted. However, none were successful. A brief discussion of the work follows; each synthetic method is treated separately.

Attempted Synthesis of a Nitro-β-hydroperoxide Ester

When 18:1 was combined with BHT and was exposed to air containing 10 ppm NO_2, a very complex product mixture resulted. After removal of the BHT and unreacted 18:1 from the product mixture, an NMR of the remaining material indicated that there was no detectable nitro compound. Although the nitro compound was probably present, the yield could have been no higher than 2%, and so the synthetic method was abandoned.

Attempted Synthesis of a Nitro-β-hydroperoxide Ester Using N_2O_4 in an Oxygen Atmosphere

A TLC separation of the product mixture from the reaction of 18:1 and N_2O_4 in oxygen produced five spots in addition to unreacted starting material. One of the spots contained a secondary nitro compound, and the nitro compound comprised about 10% of the spot (by NMR integration of the methine proton and the methyl ester protons). The attempted separation (see Experimental Section) was hampered both by the low yield of the nitro compound and by the large number of other products. Ultimately, the separation was abandoned due to the difficulties encountered.

Attempted Synthesis of a Nitro-β-alcohol Ester by Coupling of an Aldehyde with an ω-Nitro Ester

Since several couplings of a short chain primary nitro carbanion with an aldehyde to form a nitro-β-alcohol had been carried out,[101,107] it was hoped that this reaction would prove satisfactory for the synthesis of a nitro-β-alcohol ester standard. When the coupling reaction between methyl 6-nitrohexanoate and dodecanal was attempted using IRA 400 ion exchange resin as a catalyst according to the procedure of Astle and Abbott,[101] no reaction occurred at room temperature. When the reaction was repeated in refluxing ethanol, only the self-condensation product of dodecanal was isolated.

When the sodium salt of the ω-nitro carbanion was reacted with dodecanal at room temperature or in refluxing methanol, the same results were obtained.

Attempted Synthesis of a Nitro-β-alcohol by Coupling of an Aldehyde and a Primary Nitro Carbanion

The reaction of dodecanal with the carbanion of nitromethane did produce the primary nitro-alcohol in good yield (along with some self-condensation product). However, when the reaction was attempted with the 1-nitrooctane carbanion, no reaction occurred at room temperature, and only the self-condensation product was isolated from the reaction when it was repeated in refluxing tetrahydrofuran.

Attempted Synthesis of a Nitro-β-ketone Ester by Coupling of a Diester with a Primary Nitro Carbanion

When the coupling of the carbanion of 1-nitrooctane with dimethyl sebacoate was carried out at room temperature, no reaction occurred. When repeated in refluxing methanol, the reaction still gave only unreacted starting materials.

Attempted Synthesis of a Nitro-β-ketone by Coupling of an Acid Chloride and a Primary Nitro Carbanion

The carbanion of 1-nitrooctane was reacted with hexanoyl chloride in an inert atmosphere at room temperature. Only acid and 1-nitrooctane were isolated in the workup procedure. When repeated in refluxing tetrahydrofuran, the reaction gave no β-nitro-ketone.

Standardization by Comparison to a Total Nitrogen Method Using High Nitro-β-hydroperoxide Concentrations

Since standardization of the nitro method for determination of

the nitro-β-hydroperoxide concentration in autoxidation systems could not be carried out by synthesis of a model compound, the standardization is being carried out by other means. Currently, John Lightsey (of our laboratories) is standardizing the method by comparing it with a total nitrogen method (performed by Galbraith Laboratories) for samples containing high concentrations of nitro-β-hydroperoxide.[9] The extinction coefficient obtained for these samples will then be used for samples from the autoxidation systems (which contain much less nitro-β-hydroperoxide).

INTRODUCTION

The accurate determination of parts per million ozone in polluted air is one of the most difficult of all analytical problems. The extremely reactive and toxic nature of ozone, which makes accurate quantification at low levels so necessary,[1] also makes its determination extremely difficult.

Standardization of Ozone Determination Methods

In 1952, Birdsall et al.,[2] surveying the previous 50 years of literature concerning the determination of ozone, stated: "Investigations on ozone have long been hampered by the lack of a standardized method of analysis. A survey of the literature reveals a host of claims and counterclaims for various methods, mainly modifications of the iodometric method; many of these contentions have little experimental basis." Unfortunately, standardization is still one of the major difficulties encountered when developing or using an ozone determination procedure. Normally, the standardization of a determination method is carried out using a known amount of the pure species which is being determined. The obtention of pure, liquid ozone, however, is both difficult and hazardous. The vacuum transfer system required is complicated and expensive, and despite careful efforts to remove all traces of oxidizable substances, several groups have had explosions of varying intensity while attempting to work with pure ozone.[3,4] Additionally, ozone decomposes rapidly under almost all

conditions whether in the gas phase or in solution,[5-11] hence it must be determined immediately with a minimum of transfer. These dangers and problems have prevented most groups from attempting to calibrate their methods vs pure ozone.

The next most desirable method of standardization is comparison to a physical property. Although the original determination of these physical properties requires the use of pure ozone, their use for standardization does not. The physical properties of ozone have not been determined with as much accuracy as have those of most other species due to the difficulties mentioned above; however, the uncertainties in density[12] and the UV[13,14] and IR[15] extinction coefficients for ozone are less than the uncertainty of most determination methods. A physical property method, UV photometry, has recently been chosen by the EPA as the reference standardization method for all other ozone determination methods.[16] The method requires the use of special equipment, however, a multireflectance-path spectrophotometer, and to date has not been widely used even though it has been available for some time. Instead, most groups standardize their determination methods vs a wet chemical method, which was standardized vs either pure ozone or a physical property.

THE IODOMETRIC METHOD

By far the most widely used method of standardization and analysis is the neutral buffered iodometric (NBKI) method. One variation of the method[17] served as the EPA approved standardization for all other ozone determination methods, until it was superceded by a UV photometric method[16] in 1978. The iodometric method has been widely used despite

the fact that it has been surrounded by controversy since it was first used as a titrimetric method for the determination of very high (up to 25% by volume) concentration ozone.[18]

Mechanism

The iodometric method relies upon the reduction of ozone to oxygen via the concomitant oxidation of iodide to iodine (Figure I). Unfortunately, the stoichiometry is not always 1:1 as shown. A stoichiometric yield of iodine is only obtained when conditions are carefully

FIGURE I

OZONE-IODIDE REACTION MECHANISM

$$2H^+ + O_3 + 2\,I^- \longrightarrow$$

$$H_2O + O_2 + I_2$$

controlled as in the EPA approved standardization method.[17] Even the reliability of this method has been questioned,[19,20] however, and the method has been superceded as the standardization method for other methods.[16]

Stoichiometric Parameters

The stoichiometry of the iodometric method has been found to depend upon more than a dozen different parameters, many of which interact synergistically:

the concentration of the iodide trapping solution,[19-21]

the concentration of ozone being determined,[19,20]

the type of buffer used,[20-23]

the concentration of buffer,[20-22]

the type of trapping device used (frit or bubbler),[19,21,22,24]

the pH of the trapping solution,[21-23,25-29]

the acidification method, if any, including the final pH and the rate of acidification,[19,24,30,31]

the time between sampling and measurement,[19,20,22]

dust,[20-22]

the rate of sampling,[19]

iodate formation,[19,20,23,25]

hydrogen peroxide formation,[20]

and interference from other oxidants and from reductants both in the air and in the sampling system,[17,19,20,22]

Although a number of the parameters listed above are known to interact synergistically, many groups have studied a single parameter

without specifying or holding constant the other parameters. The literature concerning the iodometric method is, therefore, a mass of conflicting information. Since a discussion of the parameters individually is impossible due to their interdependence, the following discussion is chronological.

Early Titrimetric Methods

The iodometric method was first used as a titrimetric method for the determination of high (up to 25% by volume) levels of ozone.[18] In 1950, Boelter et al.[20] reviewed the work that had been done in this area. Boelter described the experiments of several groups which showed that a low pH caused a loss of iodine due to volatilization and that a high pH also caused the yield of iodine to be less than stoichiometric due to the incomplete conversion of iodate, which is formed under basic conditions, to iodine by the acidification step. He also discussed several procedures which had used a boric acid buffer rather than the more frequently used phosphate buffer. Some groups found that the use of boric acid prevented the formation of hydrogen peroxide and iodate, thus giving more accurate results, while others found that the yield of iodine was as much as 10% high when boric acid buffer was used. In his own studies, Boelter found that the yield of iodine was not dependent upon the pH over a wide range (3.5-12.3) when boric acid was used as a buffer. He determined that a lower pH caused high results and that too rapid acidification of basic samples caused loss of iodine.

In 1952, Birdsall et al.[2] reviewed the previous 50 years of literature concerning the determination of ozone, and investigated the effects of pH, boric acid or phosphate buffers, and iodide concentration.

His results were consistent with those of Boelter, with the exception of the effect of boric acid buffer. Birdsall found that boric acid gave erroneously high results. He attributed the difference in results to experimental design. In the Boelter system, the trapping solution was unstirred and the pH at the reaction interface was much higher than the bulk system. In the stirred system used by Birdsall, the pH was maintained at the buffer value.

The Saltzman Iodometric Method

Although the determination of high concentration (HC) ozone, greater than 100 µl/l, had been repeatedly investigated by the mid fifties, the determination of ozone at low concentration (LC) and at intermediate concentration (IC), 0.1-5 and 6-99 µl/l, respectively, had not been investigated. In 1959, however, B. E. Saltzman published a series of papers which described his careful investigation of the iodometric determination for HC, IC, and LC ozone.[21,22,32] This work forms the basis for all of the iodometric methods which are in use today, and for the method[17,32] which was used as the reference standardization method for all other ozone determination methods from 1959 until 1978, when it was superceded.[16]

In the first paper, Saltzman and Byers[21] investigated the photometric determination of HC ozone using a neutral 20% KI reagent and an alkaline reagent. With the former, Saltzman found curved Beer-Lambert plots and with the latter a negative intercept. He termed this negative intercept "ozone demand" and found that it increased with the age of the trapping solution.* Since neither of these methods were

*"Ozone demand" is, therefore, the amount of ozone which must be absorbed by a trapping reagent before any color development occurs.

satisfactory for the determination of HC ozone, Saltzman standardized all of his IC and LC measurements by a careful dilution of HC samples which had been quantified either by a titrimetric procedure or by UV absorbance. When Saltzman investigated the determination of IC and LC samples by both the alkaline and the neutral methods, he found a linear relationship for both methods which was independent of sample size and flow rate. When he compared these results to the standardized HC samples, good agreement was found for both in the IC range, but a divergence was found for LC samples, with the alkaline results being consistently lower. Saltzman found that, accounting for a 6% trapping loss, the neutral method gave 1:1 iodine:ozone stoichiometry for the range 2-100 μl/l. In discussion of the low results from the alkaline method, Saltzman stated that the formation of iodate or some other oxidation state of iodine could be responsible due to the loss of iodine during the acidification prior to determination.

In a second paper, Saltzman and Gilbert[22] compared the LC and IC results from the neutral and alkaline methods to another ozone determination method which formed nitrogen dioxide by the reaction of nitric oxide with ozone. The nitrogen dioxide was then determined by the Saltzman method for nitrogen dioxide.[33] The results of the nitric oxide/nitrogen dioxide method supported the contention that the neutral method gave an ozone:iodine stoichiometry of 1:1, provided the determination was carried out 45 minutes after trapping was completed to allow development of "slow color", which Saltzman had found in previous experiments as well. He found that the fraction of "slow color" to total color was reduced by sampling with a fritted bubbler rather than an impinger, and increased by an increase in the concentration of either

iodide or phosphate in the trapping solution. Increases in the concentrations of the reagents in the trapping solution also increased the total amount of color and gave a stoichiometry greater than 1:1 iodine:ozone. Further investigation of the alkaline method indicated that hypoiodite was responsible for the loss of iodine rather than iodate as had been previously postulated. Although Saltzman investigated several alkaline reagents, none eliminated this loss of iodine. Saltzman chose 1% KI in 0.1 M KH_2PO_4 and Na_2HPO_4 (his reagent number 14) as the best reagent for the determination of ozone, since it gave 1:1 stoichiometry when color was allowed to develop for 45 minutes after trapping. Saltzman postualted that there were several side reactions, some of which gave a lower and some of which gave a higher than stoichiometric yield of iodine, and that these side reactions were in balance for this reagent. Saltzman listed a number of possible side reactions, although he did not investigate the contributions from them, if any. He investigated the interference from several species known to be present in polluted air, and found that several did interfere with the determination method. He found that NO_2, SO_2, and several other oxidants and reductants interfered. He also determined that dust settling onto the glassware from the air decreased the yield of iodine appreciably. He found that iodine was lost from weak solutions of iodine even when clean glassware was used, and he postulated that such losses were responsible for the discrepancies found for samples containing low concentrations of ozone, i.e., results that were low by about 0.1 µl/l below 2 µl/l.

In the third paper, Saltzman[32] reviewed his earlier work and described the use of the neutral method in detail for use as the Public

Health Service approved method for ozone determination.

The Ingols Iodometric pH Study

In the same year, 1959, that Saltzman published his work, Ingols et al.[27] published the results of a study in which both iodine and oxygen were measured from the reaction of iodide with ozone. Ingols determined ozone concentration by the iodometric method, by oxidation of ferrous ion, and by oxidation of manganous ion in the presence of o-toluidine. Unfortunately, Ingols investigated only two pH values, 2 and 9, neither of which corresponded to the value that Saltzman recommended for analysis, 6.8-7.0. Ingols concluded that the stoichiometry (iodine:ozone) was 1:1 at a pH of 9 and that it was higher at pH 2. He also concluded, as had Saltzman, that the stoichiometry was balanced by several competing side reactions. The difficulties encountered in the iodometric method caused Ingols to suggest the use of the manganous/o-toluidine system for the determination of ozone.

The Cohen, Purcell, and Altshuller Interference Study

Cohen, Purcell, and Altshuller[34] reviewed the iodometric method in 1967, and compared it with several other procedures including one that they had developed. They determined the amount of I_2 liberated per mole by a variety of peroxides, peracids, and NO_x (Table I). As can be seen from the table, most peroxy compounds interfere strongly since they, like ozone, produce about 1 mole of I_2 per mole. Indeed an iodometric method is used for the determination of organic peroxides.[35] Thus the iodometric method is satisfactory for the determination of ozone only in the absence of other oxidizing or reducing species.

TABLE I

Interferent	Molar Absorptivity
Peracetic Acid	26,200
Hydrogen Peroxide	24,000
Acetyl Peroxide	24,000
Ethyl Hydroperoxide	20,200
n-Butyl Hydroperoxide	43,300
NO_x	2,400

The Boyd Stoichiometry Challenge

In 1970, Boyd, Willis, and Cyr[25] published a very controversial paper challenging the findings of Saltzman.[21,22,32] Boyd concluded, based on comparison of the NBKI method with UV photometry, that more than one mole of iodine was liberated from a mole of ozone. He found the stoichiometry to be 1.54:1.00 iodine:ozone at pH 7.0. Boyd further concluded that the results of Ingols[27] were in conflict with those of Saltzman and in agreement with his.

Refutation of Boyd and Support for the NBKI Method

A series of papers were published, chiefly by EPA scientists, in response to the paper by Boyd et al.[25] All of these papers, which re-investigated the stoichiometry of the neutral iodometric method, supported the original findings of Saltzman,[21,22,32] although none were able to find an error in the work carried out by Boyd.

Hodgeson et al.[28] reinvestigated the Saltzman method and its

comparison to the nitric oxide conversion method.[22] They stated that the conversion yield of nitrogen dioxide from nitric oxide was 0.72, and appear to have determined this by comparison to an ethylene chemiluminescence method. They did not state the method by which the chemiluminescent method was calibrated, however, and thus the validity of their support for the Saltzman method is somewhat questionable.

Kopczynski and Bufalini[26] compared the Saltzman method to a long path IR method. Within experimental error, their results were in agreement with those of Saltzman; i.e., the stoichiometry found was 1:1. The authors also cited several investigations of reactions between olefins and ozone in which the stoichiometry had been determined by comparison to the iodometric method. The authors stated that the 1:1 stoichiometry found for ozonolysis of these olefins, by comparison to the iodometric method, was support for the conclusion that the stoichiometry of the iodometric method was 1:1.

The Iodometric Standardization Reference Method

In 1971, after investigating the claims of Boyd et al.,[25] the EPA published an approved neutral buffered iodometric method for the determination of ozone entitled: "Reference Method for the Measurement of Photochemical Oxidants Corrected for Interference Due to Nitrogen Oxides and Sulfur Dioxide."[17] The method was an adaptation of the original method published by Saltzman for the then Public Health Service,[32] with careful specification of the parameters known to affect the determination. One of the major differences was the elimination of delay between sampling and measurement. Despite the difference, the stoichiometry for the method was stated to be 1:1 iodine:ozone.

This method served as the reference method for the determination of ozone for many years, and has only recently been superceded by a UV photometric procedure.[16]

Many groups have investigated various facets of this method. For example, Parry and Hern[24] studied the formation of iodate. Parry found that there was little iodate formed when measuring LC samples of ozone. However, when HC ozone was determined, iodate formation became important. The formation of iodate at HC was found to be catalyzed by the use of borosilicate or quartz frits in the trapping system, and minimized by the use of an impinger. Parry postulated that the stoichiometric variations found by earlier workers might have been due to the formation of iodate. He suggested a simple test for iodate formation, i.e., a sample was determined, acidified, and determined again. If additional iodine formed after acidification, iodate was present. Parry stated that the formation of iodate was important not only for HC samples under basic conditions, as had been postulated by Saltzman, but also for neutral conditions if a catalyst was present. A communication by Schmitz[30] and the reply of Parry[31] state that acidification immediately before titration prevents losses of iodine and provides a 1:1 stoichiometry for HC samples under all conditions.

In 1973, Dietz, Prezansky, and Smith[29] reinvestigated the effects of pH on the stoichiometry of the iodometric method, calibrating their studies vs UV photometry. Smith found the stoichiometry to be 1:1 for the neutral method over the range 50-600 µl/l ozone. He also found significant losses of ozone when a single impinger was used for trapping HC ozone, but no significant losses for IC and LC ozone.

Goates, Bradshaw, and Mangelson[36] designed a modification of the

neutral buffered iodometric method which allowed the determination of ozone down to about 5 nl/l. In the procedure, starch was added to the trapping solution to increase the absorbance, and hence the sensitivity of the determination. Mangelson found that the method reliably determined ozone in the range 5 nl/l-1 µl/l by comparing it to a Mast ozone meter method. Unfortunately, Mangelson did not investigate the effects of other parameters on the method.

The Flamm Investigation of the NBKI Method

In 1975, Flamm and Anderson[19] published a paper which described a careful investigation of the effects of four variables: pH, ozone concentration, elapsed time between sampling and analysis, and the use of impingers of fritted bubblers on the determination of IC and HC ozone by the neutral buffered iodometric method.

In review of previous work, Flamm noted that Saltzman[21,22] had found that the LC and IC results from the Saltzman method were low compared to HC results, and that Saltzman had used midget impingers. He also noted that Saltzman had found that additional color developed after sampling had been terminated, and that the stoichiometry was 1:1 only when absorbance was measured 45 minutes after sampling. Flamm pointed out that the EPA approved method,[17] which was based on the Saltzman method, specified that measurement be made immediately after sampling, yet still claimed 1:1 stoichiometry. Flamm also pointed out that most groups who had investigated the iodometric method had not specified the elapsed time between sampling and analysis at all.

In his own investigations, Flamm found that decreasing the pH caused a decrease in the formation of iodate, and that the formation

of iodate was significant for HC samples even at neutral pH. He concluded that iodate was being formed in the reaction by comparing the rate of decomposition of the material formed in the trapping solution to the rate for authentic iodate. Parry[24] had concluded that iodate was formed by comparing the half-wave potentials of the trapping solution to an authentic sample of iodate. Flamm found, as had Parry, that the amount of iodate formed decreased with decreasing ozone concentration. Flamm postulated that many of the discrepancies found in the literature of the iodometric method could be due to differences in elapsed time between sampling and analysis, since the iodate formed reacts to produce additional iodine after sampling is terminated. Flamm concluded, as had Parry[31] and Schmitz,[30] that acidification immediately prior to analysis was satisfactory for the determination of IC samples. However, Flamm suggested that this procedure be used only for samples of more than 50 µl/l ozone. He stated that, below this level, the oxidation of iodide to iodine in the trapping medium would produce erroneously high results.

In his most recent paper, Flamm[20] studied the determination of LC ozone in air. He carefully investigated the EPA approved adaptation of the Saltzman method[17] as well as several variations of the method. Flamm determined that the use of a phosphate buffer catalyzes the formation of another product, hydrogen peroxide, in addition to iodate. He concluded that hydrogen peroxide was the product formed by comparing the reaction rates of the trapping product and authentic hydrogen peroxide. Flamm determined that a decrease in the concentration of phosphate decreased the final amount of hydrogen peroxide, although high concentrations of buffer gave lower initial amounts of hydrogen peroxide.

Flamm investigated several other buffer systems, principally boric acid systems and sulfuric acid systems, and found that none of these produced hydrogen peroxide. Since the hydrogen peroxide formed from phosphate buffered systems reacts with iodide to form additional iodine after sampling is terminated, the extent of the nonstoichiometric behavior of the method depends upon the elapsed time between sampling and analysis. Flamm determined that a 2-minute delay produced about a 20% increase beyond 1:1 stoichiometry for the NBKI system for ozone samples below 1 µl/l. Flamm also investigated the effect of water purity on the iodometric method and found that distilled water or any higher grade gave identical results. In his investigation of "ozone demand", Flamm found a significant demand for samples containing less than 0.1 µl/l ozone. He also determined that the amount of "ozone demand" for a particular reagent decreased with the age of the reagent, and that it was greater for potassium iodide of lesser purity. Flamm postulated that the "ozone demand" is responsible for the low yield of iodine from the boric acid buffered samples at less than 0.1 µl/l, and suggested that the addition of hydrogen peroxide to the reagent before use could eliminate the demand without creating unacceptably high blanks. Flamm concluded that, using phosphate buffer, the iodometric method had two balancing effects: "ozone demand" and the formation of hydrogen peroxide. According to Flamm, the former produces low results from 0.35-1.00 µl/l, the latter balances its effect from 0.10-0.35 µl/l, and below 0.10 µl/l, the scatter of the method is most important.

OTHER DETERMINATION METHODS

The Ethylene Chemiluminescence Method

The failings of the iodometric method have prompted many groups to develop other ozone determination methods. The method most widely used for the determination of ozone in polluted air is the ethylene chemiluminescence method,[17] which is the method currently approved by the EPA. The method requires the use of a specific instrument, however, which is not commonly found in laboratories, and also is subject to interference by quenching. Although it has gained wide acceptance for field studies, the ethylene chemiluminescence method is not widely used in laboratory analysis.

Nitrogen Dioxide Methods

Numerous groups have developed determination methods which rely upon the reaction of nitric oxide with ozone to produce nitrogen dioxide, which is then determined by the Saltzman method,[37] by chemiluminescence,[38] or by a nitrate specific-ion electrode.[39] These methods, however, have not enjoyed wide acceptance. They are difficult, requiring careful metering of the reactant gasses, and are subject to interference from nitrogen dioxide in air.[40]

Mast (Electrochemical) Ozone Meters

An instrumental method for the determination of ozone was developed by Wartburg et al.[41] This ozone monitor, the Mast ozone meter, determines ozone by measuring the current required to reduce a KI/KBr mixture which is continuously oxidized by an air stream containing ozone. Unfortunately, the method is subject to most of the failings of the

iodometric method on which it is based, and the stoichiometry of the method varies with ozone concentration.[34] Attempts have been made to improve the method, notably by the use of catalysts which decompose ozone.[40] The current is measured first with the catalyst in stream and then with it out of stream, and the ozone concentration is determined as the difference between the two signals. These methods do not eliminate the difficulties produced by variable stoichiometry, however, and are not widely used.

Miscellaneous Methods

One group attempted the determination of ozone by the oxidation of 9,10-dihydroacridine followed by the spectrophotometric determination of the oxidized product.[42] The method is subject to interference from other oxidants, however, and involves the use of a fluorophotometer.

A very novel approach was taken by Chleck et al.[43,44] They prepared a clathrate of Kr^{85} in quinol (hydroquinone) which released Kr^{85} upon exposure to ozone. The Kr^{85} was then determined in the effluent stream. Although the method requires special equipment and is subject to interference from other oxidants, it provides excellent sensitivity, parts per ten billion.

Bradley and Haagen-Smit[45] suggested a qualitative method which detects ozone by observing the incidence of cracking in stressed rubber exposed to air containing ozone. Although fairly sensitive, the method is nonspecific.

In 1966, Hendricks and Larsen[46] reviewed several methods for the determination of ozone. They found the iodometric method of Saltzman,[32] the phenolphthalein method of Haagen-Smit and Bruenelle,[47] the sodium

diphenylamine sulfonate method of Bovee and Robinson,[48] and the leucofluorescein method of Egorov[49] to be subject to interference from other oxidants in polluted air and hence not acceptable for ozone determination in air.

Cohen, Purcell, and Altshuller[34] also reviewed a number of methods and found that the UV photometric method and the Mast coulometric method[41] are subject to interference from a number of species found in polluted air. They also investigated two redox methods, using ferrous thiocynate and titanium-8-quinolinol, respectively. These methods were found to lack specificity.

Stevens and O'Keeffe[50] described a chemiluminescent ozone determination procedure which determines ozone via a chemiluminescent reaction with Rhodamine B absorbed on silica gel. Although sensitive, the method is subject to interference from other oxidants.

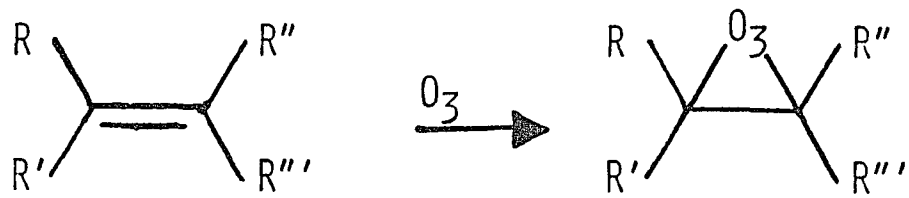
OZONOLYSIS METHODS

Mechanism

Several groups have developed ozone determination methods which are based on the only reaction unique to ozone, ozonolysis. When ozone reacts with a double bond, a molozonide, (I, Figure II) is produced. The molozonide then decomposes either via a peroxide zwitterion and a carbonyl fragment (Schemes I and II, Figure III) or via a pair of diradicals (Schemes III and IV, Figure IV). Recombination can then occur to form any of three Criegee ozonides (I, II, and III, Figure V). In some cases the molozonide can be isolated at low temperatures,[51] but

FIGURE II

Molozonide Formation



(I)

under normal conditions it decomposes immediately. The Criegee ozonides can decompose in the same manner as the molozonide, and the intermediates formed can react with other species present in the system to give a variety of products.[52-57]

Determination of Ozone by Quantification of the Carbonyl Fragment from Ozonolysis

Most groups have carried out their determinations by measuring the carbonyl fragments (II and III, Figure III) formed from the decomposition of the ozonides. Bravo and Lodge[58] determined ozone via the ozonolysis of 4,4'-dimethoxystilbene to produce 4-methoxybenzaldehyde which

FIGURE III

MOLOZONIDE DECOMPOSITION (IONIC)

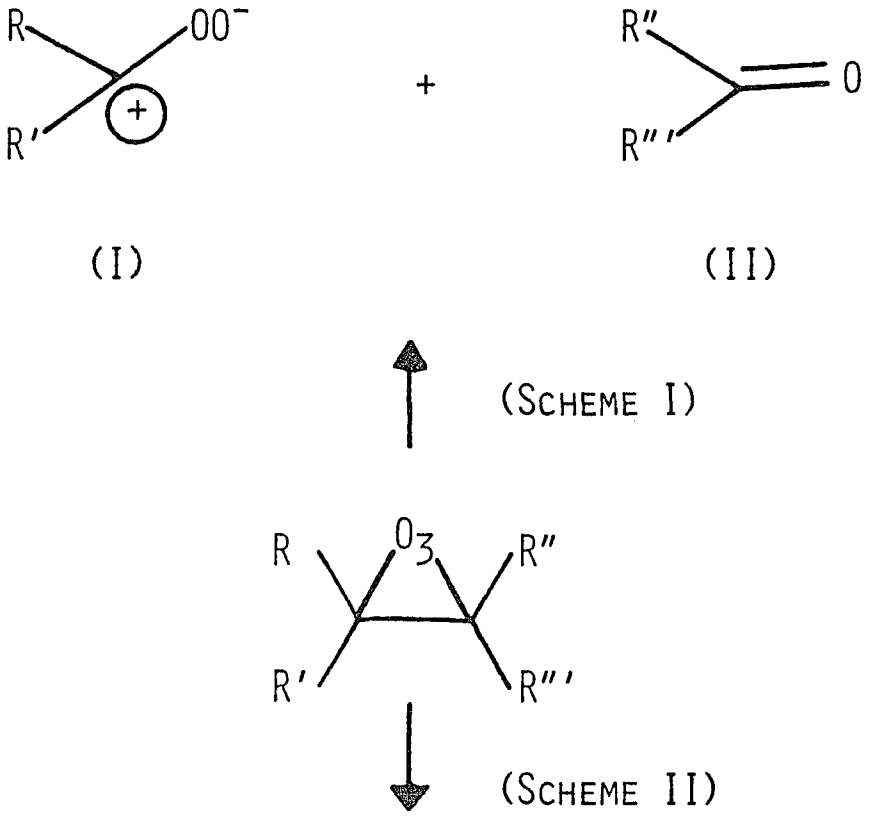
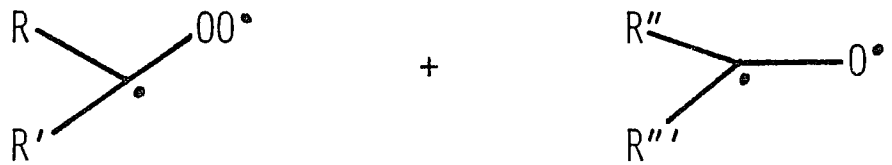


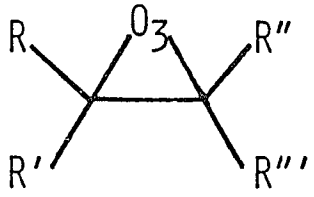
FIGURE IV

MOLOZONIDE DECOMPOSITION (RADICAL)

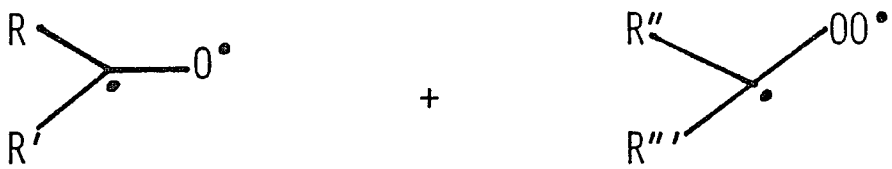


(IV) (V)

(SCHEME III)



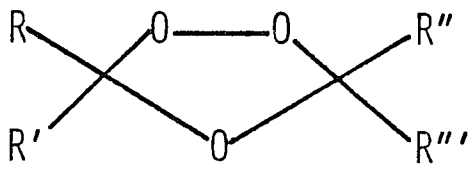
(SCHEME IV)



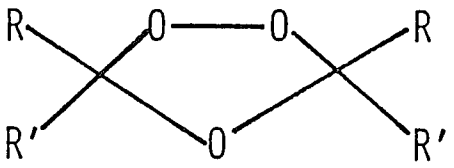
(VI) (VII)

FIGURE V

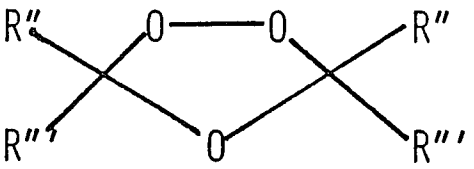
CRIEGEE OZONIDES



(I)



(II)



(III)

was determined by the procedure of Sawicki.[59] The yield of 4-methoxybenzaldehyde (anisaldehyde) from the reaction was 98%. Unfortunately, the aldehyde remains in the trapping solution in this method, and is subject to further oxidation by ozone. Also the method is subject to interference from other oxidants such as NO_x and organic peroxides. These species probably interfere via a free radical initiated cooxidation.[60] Finally, the reaction requires the use of trifluroacetic acid, which is extremely corrosive, hence the method has not found wide use.

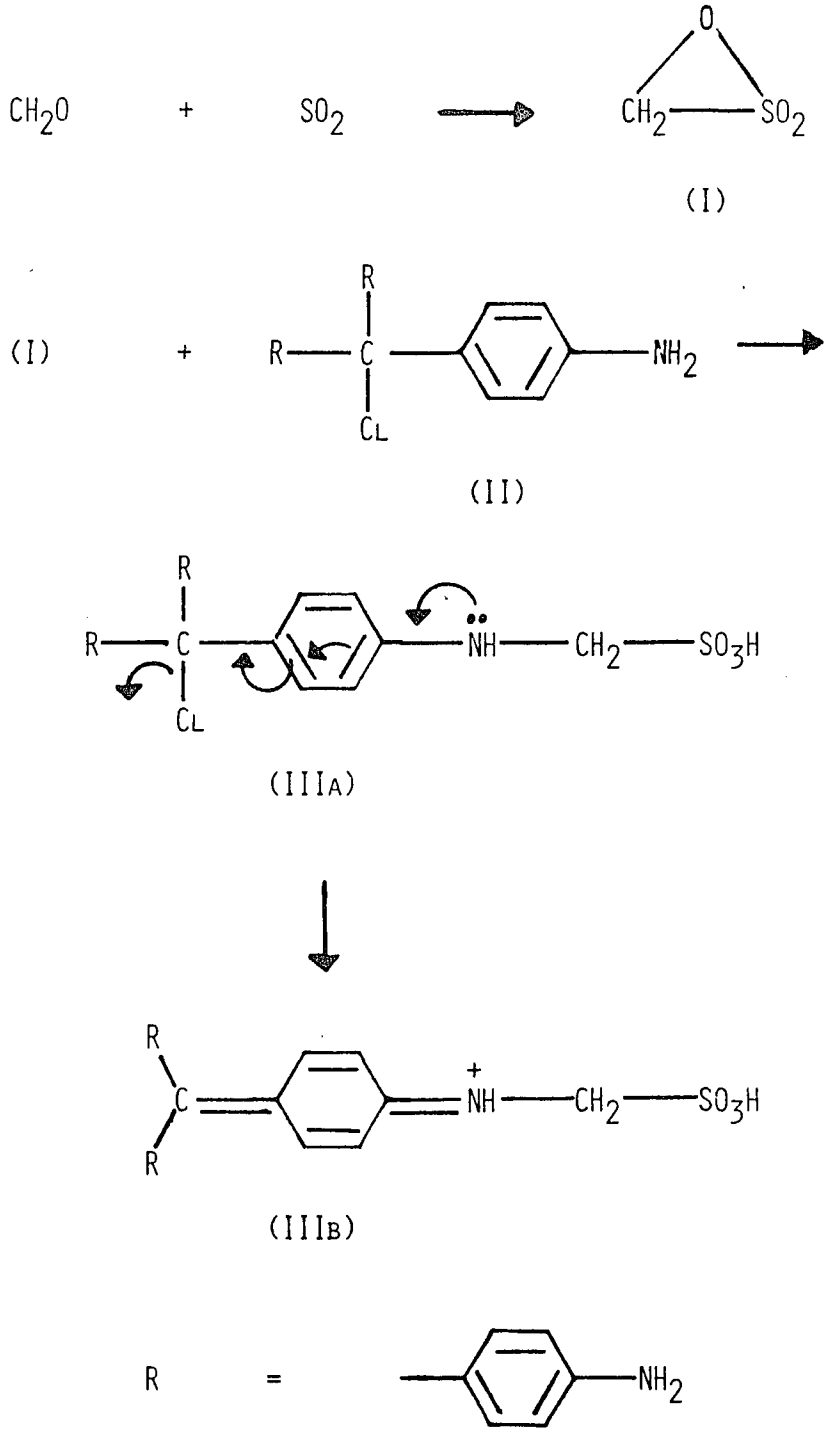
Hauser and Bradley[61] developed a similar method based on the ozonolysis of 1,2-di-(4-pyridyl)ethylene. Their method is subject to the same problems, however, in that other species interfere, the aldehyde is subject to further oxidation, and a strong acid, glacial acetic, is used in the determination. In a second paper,[62] Hauser and Bradley investigated the effects of several interfering species on their method and found significant interference from peroxyacetyl nitrate (PAN) and hydrogen peroxide, as well as lesser interference from a number of other species.

The West Method (Advantages of Formaldehyde)

In 1972, Sachdev, Lodge, and West[63] developed a method which overcame some of the failings of earlier ozonolysis determination methods. West carried out the determination of ozone by ozonizing eugenol (4-allyl-2-methoxyphenol) to produce formaldehyde. The formaldehyde was then blown out of the eugenol and into a second bubbler containing water where it was trapped. West determined the formaldehyde produced by the reverse West-Gaeke method of Lyles, Dowling, and Blanchard (Figure VI).[64]

FIGURE VI

WEST-GAEKE SO_2 OR REVERSE WEST-GAEKE CH_2O METHOD



By blowing the formaldehyde out of the eugenol and into a second bubbler, West protected it from further oxidation ensuring a more accurate determination. West found that the yield of formaldehyde from his method was equivalent, within experimental error, to the amount of iodine produced from the alkaline iodometric method.[32] He mentioned that Boyd[25] had recently stated that the stoichiometry of the alkaline method was 1:1, and that Kopczynsci and Buffalini[26] had challenged this statement. West concluded that the stoichiometry of his method could not be determined without a means of standardization, but that it was consistent with a 1:1 stoichiometry for the alkaline iodometric method. The stoichiometry of the alkaline method has since been shown to be closer to 1.54:1.00 iodine:ozone, and thus the yield of formaldehyde from the ozonolysis of eugenol is 67%.

West also investigated interference from hydrogen peroxide and from peracetic acid. He found no interference from either, when solutions of either were sprayed into the air being sampled. Although not discussed by West, this freedom from interference, which was not found for other ozonolysis methods, is almost certainly due to the presence of the phenolic group in eugenol. The phenol sacrificially protects the olefin from free radical initiated oxidation, which had been shown to interfere with the method of Hauser and Bradley.[62]

Although better than previous methods, the method of West still has limitations. The yield of formaldehyde was 33% below theoretical, and although the phenolic group did protect the system from interference by free radical initiators, it may have competed with the olefin for reaction with ozone, thus decreasing the yield of formaldehyde.

I, therefore, set out to develop a method which would retain the

advantages of the West method without being subject to its disadvantages.

Factors Affecting the Yield of Formaldehyde from the Ozonolysis of a Terminal Olefin

The ionic decomposition of an ozonide can proceed in two ways (Schemes I and II, Figure III). The production of formaldehyde from a terminal olefin via ionic decomposition (Scheme I, R" = R"' = H) is aided by stabilization of the zwitterionic intermediate (I, Figure III), either by resonance or by the electron donating abilities of R and R'. Thus the ozonolysis of a tertiary terminal olefin, which produces a tertiary carbocation, would be expected to give a higher yield of formaldehyde than that from the ozonolysis of eugenol, which forms a secondary zwitterion. Indeed, the ozonolysis of 2,2,4-trimethylpentene has been shown to give a quantitative yield of formaldehyde,[65] while West found a 67% yield of formaldehyde from eugenol.

Hammett Studies of Styrenoid System Ozonolysis

Several groups have investigated the effects of resonance and induction on the ozonolysis of styrenoid molecules.[52,66,67] As would be predicted if a zwitterion is formed, these groups found that the yield of formaldehyde was greatest when electron donating groups were substituted onto the ring.[66,68] For example, Flizar and Granger[66] found a 60% yield of formaldehyde from the ozonolysis of styrene, and an 80% yield from p-methoxy-styrene. Thus, a yield of formaldehyde greater than the 67% found by West [63] is possible when one takes advantage of both resonance and inductive effects. I, therefore, studied the

ozonolysis of a number of commercially available styrene derivatives, in order to determine which of them was best suited to the determination of ozone, by virtue of giving the greatest yield of formaldehyde with a consistent stoichiometry.

Formaldehyde Determination Methods

I investigated several of the literature methods for the determination of formaldehyde. The chromatrophic acid method[69] had been studied by West,[63] who found it unsuitable due to interference from olefin carried over into the formaldehyde trap and undesirable due to the use of concentrated sulfuric acid in the formaldehyde determination procedure. Several formaldehyde determination methods using 3-methyl-2-benzothiazolone hydrazone have been suggested,[70-73] however, these methods are undesirable since they require the synthesis of the trapping reagent. The p-phenylenediamine method of Bailey and Rankin[74] was eliminated because it uses concentrated hydrogen peroxide. Two gas chromatographic methods were considered,[75,76] but these were discarded as difficult and likely to be subject to interferences. Ultimately, the formaldehyde determination method used by West[64] was chosen as the most satisfactory. This method, developed by Lyles, Dowling, and Blanchard,[64] determines formaldehyde by the reverse of the West-Gaeke method for the determination of sulfur dioxide. Nauman, West, and Tron[77] have shown that these two methods determine formaldehyde or sulfur dioxide, respectively, by their reaction to form an adduct (I, Figure VI). This adduct then reacts with pararosaniline (II, Figure VI) to form a substituted aniline derivative (III, Figure VI) which has an absorption maximum between 540 and 580 nm, depending upon the pH. The lesser

basicity of this substituted aniline compound precludes the protonation of the nitrogen, and consequently allows the formation of the 540-580 nm chromophore at a pH sufficient to protonate, and decolorize, all of the unreacted pararosaniline. A measure of the amount of this chromophore is, therefore, a measure of the amount of sulfur dioxide in the presence of excess CH_2O (the West-Gaeke method) or of formaldehyde in the presence of excess SO_2 (the reverse West-Gaeke method).

Standardization

The Lyles formaldehyde determination method[64] was standardized with commercial formaldehyde, the concentration of which was determined by the sulfite method.[78] In this procedure, formaldehyde is reacted with sodium sulfite in water to produce an equimolar amount of hydroxide (Figure VII), which is then determined by titration with standard acid.

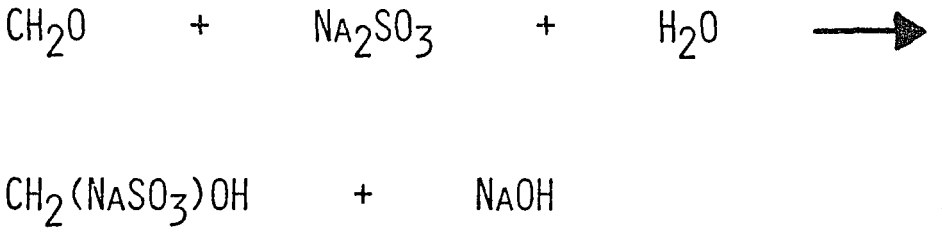
Standardization of the olefin method was carried out by comparison to the NBKI method.[17] The UV photometric method,[16] which has recently superceded the NBKI method as the EPA approved reference standardization for ozone determinations, had not been published by the time this work was completed.

Free Radical Inhibitors

Lastly, several free radical inhibitors were investigated in order to find one which would protect the olefin from free radical oxidation without competing with olefin for reaction with ozone.

FIGURE VII

Mechanism of Formaldehyde-Sulfite Reaction



MATERIALS AND EQUIPMENT

Pararosaniline acetate was obtained from Eastman Kodak, and was used without further purification.

Water was distilled, deionized by passage through a mixed bed column, and boiled.

Mesitol (2,4,6-trimethylphenol) was obtained from Aldrich Chemical Co., and was used without further purification.

Eugenol (4-allyl-2-methoxyphenol), 2-Vinylnaphthalene, 4-Vinylanisole, and DPE (1,1-diphenylethylene) were obtained from Aldrich Chemical Co., and were purified before use according to the procedure described in the ozone determination procedure.

All other chemicals were reagent grade or better.

Ozone germicidal lamps were obtained from General Electric, GE 4W-S11.

Flowmeters were obtained from the Fisher and Porter Corp., No. 448-118, and were accurate to within 2%.

The spectrophotometer used was a Beckman DU equipped with Gilford power supply, sample changer, and detector.

All volumetric glassware was Class A.

Midget impingers were obtained from Ace Glass, Inc., No. 7531 Code 10.

Fritted bubblers were obtained from Ace Glass, Inc., No. 7530 Code 05.

EXPERIMENTAL PROCEDURES

OZONE GENERATION AND DETERMINATION APPARATUS

Ozone Generator

Tank breathing air is passed over activated charcoal and silica gel and then passed into an all glass and Teflon ozone generating chamber which contains four 4 watt germicidal lamps (Figure VIII). The ozone output is adjusted by varying the number of lamps and by varying the voltage to the lamp(s) with a variable voltage transformer. The ozonized air is then passed through all glass apparatus, with ground glass or Tygon butt joints, to the trapping devices, and then through the flowmeter.

Detection Apparatus

When determined by the iodometric method, the ozone stream is trapped in a fritted bubbler containing 10 ml of iodometric trapping reagent.

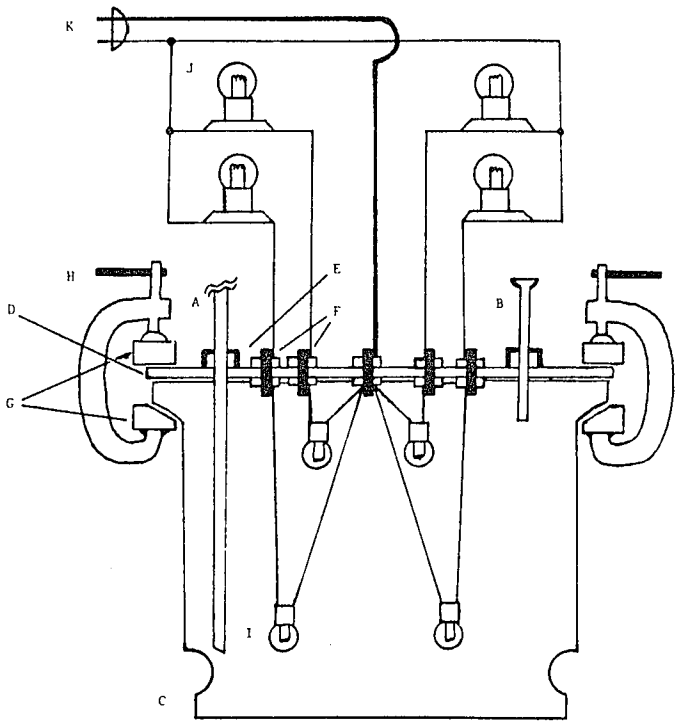
When determined by the olefin method, the ozone stream is impinged on 1 ml of the olefin, and the effluent from the impinger is trapped in 10 ml of water contained in a fritted bubbler.

THE IODOMETRIC METHOD[17]

Standardization of Iodine Solution

FIGURE VIII

Ozone Generating Apparatus



A. Purified Air Inlet
B. Ozonized Air Outlet (To Trapping Apparatus)
C. 250 cm Dessicator Base
D. 1/4 in. Teflon Plate
E. 3/8 in. Kelvar Fittings
F. Threaded Brass Rods (With Nuts on Each End)
G. Aluminum Rings (Lower Ring Beveled to Fit Dessicator
H. C Clamps (8 Equidistant Around Rings)
I. 4 Watt Germicidal Lamps (GE 4W-S11)
J. 40 Watt Ballast Lamps
K. AC Line to Variable Transformer

Pipet accurately 20 ml of standard arsenious oxide solution into a flask. Acidify slightly with 1:10 sulfuric acid, neutralize with solid sodium bicarbonate, and then add about 2 g of excess bicarbonate. Titrate with the standard iodine solution using 5 ml starch solution as an indicator. Saturate the solution with carbon dioxide near the end point by adding 1 ml of 1:10 sulfuric acid. Continue the titration to the first appearance of a blue color which persists for 30 seconds.

Preparation of a Standard Curve

Immediately before use, pipet 1 ml of standard iodine solution into a 100 ml volumetric flask and dilute to volume with absorbing reagent. Into a series of 25 ml volumetrics, pipet 0.5, 1, 2, 3, and 4 ml of the diluted standard iodine solution. Dilute the contents of each flask with absorbing reagent, mix throughly, and immediately read the absorbance of each at 352 nm vs unexposed absorbing reagent.

Analysis of Air Samples

Pipet 10 ml of the absorbing reagent into the trapping bubbler, and draw air through it at 200-1000 ml/minute. Quantitatively transfer the trapping solution to a 25 ml volumetric and dilute with absorbing reagent. If necessary, dilute an aliquot with additional absorbing reagent. Determine absorbance at 352 nm immediately, using unexposed absorbing reagent as reference.

REAGENTS:

Absorbing Reagent: Potassium dihydrogen phosphate, 13.6 g,

disodium hydrogen phosphate, 14.2 g, and potassium iodide, 10.0 g, are dissolved in water and diluted to exactly 1 liter. This solution should have a pH of 6.8 $\pm$ 0.2, and is stable for several weeks if stored in a glass stoppered bottle in a cool dark place.

Standard Arsenious Oxide (As_2O_3) Solution: Primary standard arsenious oxide is dried for 1 hour at 105°C, cooled, and 2.4 g is accurately weighed into a beaker. Sodium hydroxide, 25 ml of a 1 N solution, is added, the beaker is heated to dissolve the As_2O_3, and 25 ml of 1 N H_2SO_4 is added. The beaker is cooled, its contents are transferred quantitatively to a 1 liter volumetric, and the contents are diluted to the mark.

Starch Indicator Solution (0.2%): Soluble starch, 0.4 g, is mixed with about 2 mg of mercuric iodide (as a preservative) in a little water. The paste is slowly added to 200 ml of boiling water, and the solution is boiled until it clears. The solution is then cooled, and transferred to a glass stoppered bottle.

Standard Iodine Solution (0.05 N): Potassium iodide, 5.0 g, and iodine (resublimed), 3.2 g, are dissolved in 10 ml of water. When the iodine dissolves, the solution is transferred to a 500 ml volumetric and the contents are diluted to the mark with water. This solution must be stored in the dark, and must be restandardized as necessary.

STANDARDIZATION OF COMMERCIAL FORMALDEHYDE[78]

Sodium sulfite solution, 50 ml, and 3 drops of thymolphthalein indicator solution are added to a flask and carefully neutralized by titration with 1 N sulfuric acid until the blue color disappears (one or

two drops). A neutral formaldehyde sample is then added to the flask and the resulting solution is titrated slowly to complete decolorization using the standard sulfuric acid.

REAGENTS:

Sodium Sulfite Solution: Sodium sulfite, 126 g, is dissolved in exactly 1 liter of water.

Thymolphthalein Indicator Solution: Thymolphthalein indicator is 0.1% in ethyl alcohol.

Standard Sulfuric Acid (1 N): Sulfuric acid, 1 N, is standardized vs 1 N NaOH, which is in turn standardized vs potassium acid phthalate.

FORMALDEHYDE DETERMINATION METHOD[64]

Preparation of Standard Curve

Using a standard solution of formaldehyde (prepared by a suitable dilution of the standard above), prepare a series of standards in 25 ml volumetrics. Add sufficient standard solution to achieve a range of final concentrations between 0.1-1.0 µg/ml. To the volumetrics add 1 ml of the sodium dichlorosulfitomercurate (II) reagent, and mix thoroughly. Add 2 ml of the pararosaniline reagent, mix thoroughly, dilute the contents, and allow to stand for 25 minutes. Then determine the absorbance at 580 nm vs a water reference.

Sample Determination

Air containing formaldehyde is trapped in 10 ml of water contained in a fritted bubbler. After trapping, the contents of the bubbler are quantitatively transferred to a 25 ml volumetric, and the sample is determined according to the procedure used for formaldehyde standards. If necessary, the volumetric is filled to the mark with water, and an aliquot is taken for determination.

REAGENTS:

Sodium Tetrachloromercurate (II): Add 27.2 g of mercuric chloride and 11.6 g of sodium chloride to a 1 liter volumetric, dissolve the salts, and dilute the contents of the flask to the mark with water.

Sodium Dichlorosulfitomercurate (II): Add 0.25 g of sodium sulfite to 25 ml of the sodium tetrachloromercurate (II) reagent, and mix thoroughly. This reagent is unstable and must be prepared fresh every 24 hours. Also, mercury salts are poisonous, and care should be taken in the handling of these solutions.

Pararosaniline Reagent: Add 0.448 g of pararosaniline acetate to 67.5 ml of concentrated hydrochloric acid in a 250 ml volumetric, and dilute to volume.

DPE OZONE DETERMINATION PROCEDURE

DPE (1,1-diphenylethylene), 1 ml, is placed in a midget impinger, and 0.1 ml of mesitol (2,4,6-trimethylphenol) solution is added. The

impinger is then placed in the ozone trapping train, consisting of the impinger, followed by a bubbler containing 10 ml of water, followed by a flowmeter accurate to within 2%. A stream of air containing ozone is then blown through the apparatus at a known rate for a known time, the bubbler is removed, and the amount of formaldehyde is determined according to the formaldehyde determination procedure described above. A blank, obtained by sampling the air stream without the impinger in the trapping train, is subtracted from each value.

REAGENTS:

DPE: DPE contains small amounts of formaldehyde which are removed immediately before use either by passing the DPE through a plug of sodium sulfite or by blowing air or nitrogen through the impinger containing DPE for 10 minutes before placing the impinger in the trapping train.

Mesitol Solution: The mesitol solution is 2 g of mesitol in 10 ml of xylene.

STOICHIOMETRY STUDIES

The stoichiometries of the olefin-ozone reactions were determined by first determining the ozone output via the iodometric method, and then determining the yield of formaldehyde via the DPE procedure.

INHIBITOR STUDIES

Free radical inhibitor studies were carried out according to the DPE procedure with various inhibitors substituted for mesitol.

NO_2 INTERFERENCE STUDIES

The NO_2 interference studies were carried out according to the DPE procedure, with the addition of an NO_2 permeation tube[3] to the ozone generating chamber.

RESULTS AND DISCUSSION

FORMALDEHYDE DETERMINATION

Standardization of Commercial Formaldehyde

When standardized by the sulfite method[78] (see Experimental Section), commercial formaldehyde (37% in water with methanol added as a stabilizer) was found to contain 36.29% (389.2 mg/ml) formaldehyde. The reproducibility of this determination was excellent, 2 parts per thousand. However, care must be taken to ensure that the formaldehyde being standardized is neutral or to account for its acidity by titration of a sample with standard base and addition of this blank to the amount of base determined by the sulfite method.

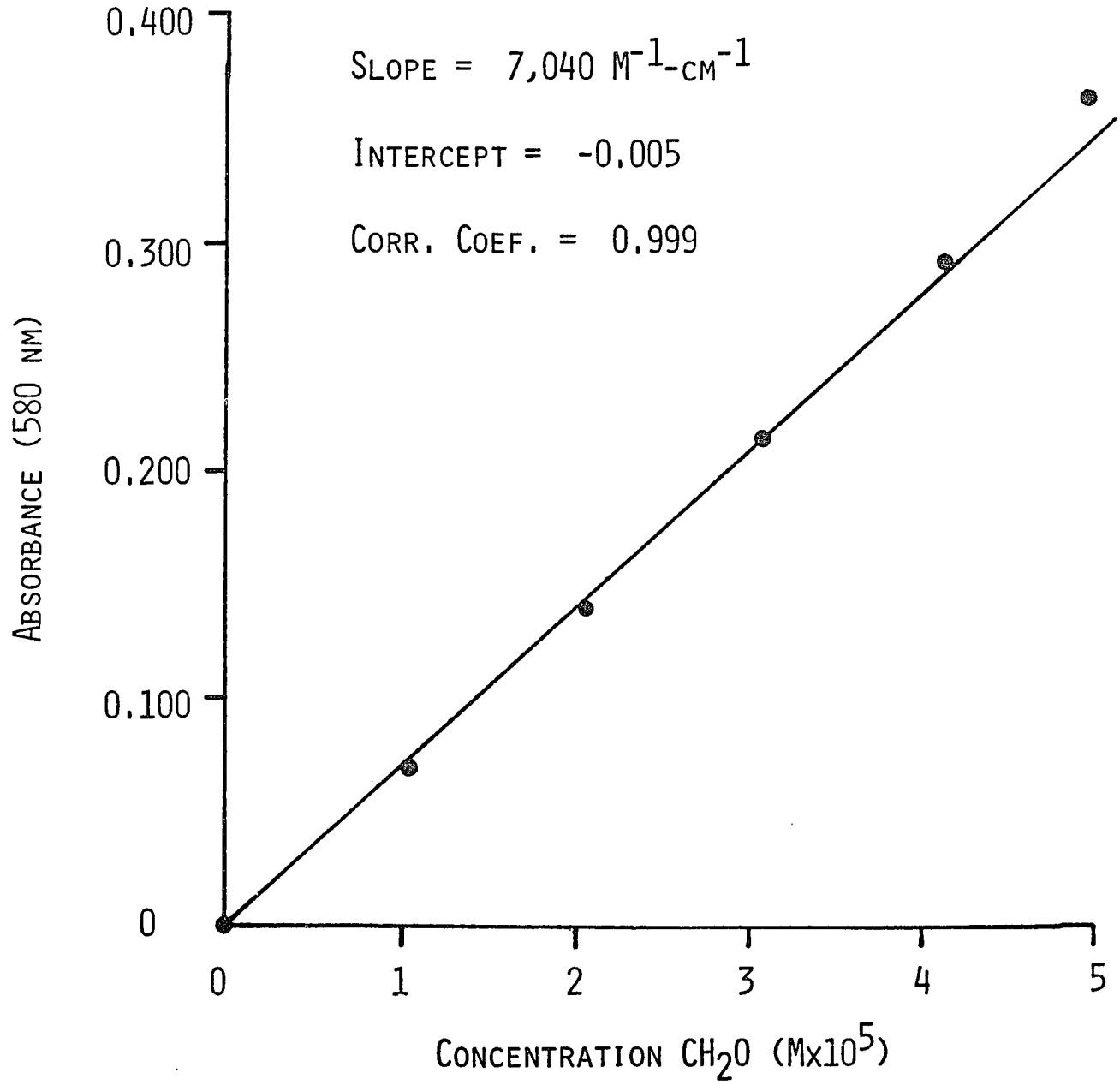
Preparation of Standard Curves

When an aliquot of the standard formaldehyde solution was diluted and used to prepare a standard curve according to the pararosaniline procedure (see Experimental Section), a linear Beer-Lambert plot was obtained with positive deviation above 4×10^{-5} M CH_2O (Figure IX). The extinction coefficient obtained was 7,040 M^{-1}-cm^{-1} $\pm$ 220.* When standardization of a fresh set of reagents was carried out 1 month later, an extinction coefficient of 6,880 $\pm$ 160 M^{-1}-cm^{-1} was obtained. Subsequent restandardizations of either reagent agreed with the original

*Extinction coefficients were determined by linear regression, intercepts and correlation coefficients are given with each plot.

FIGURE IX

ABSORBANCE (580 NM) VS CONCENTRATION OF FORMALDEHYDE



value to within 4%. The reagent solutions, sodium tetrachloromercurate (II) and pararosaniline, were stable for at least 9 months, as had been found by Saltzman.[79]

Blank Determination

Although formaldehyde blanks from freshly boiled water were consistent within 2% over a period of several months, blanks from routine analyses varied, primarily with the age of the water used for dilution. Therefore, a blank was determined with and subtracted from each set of samples.

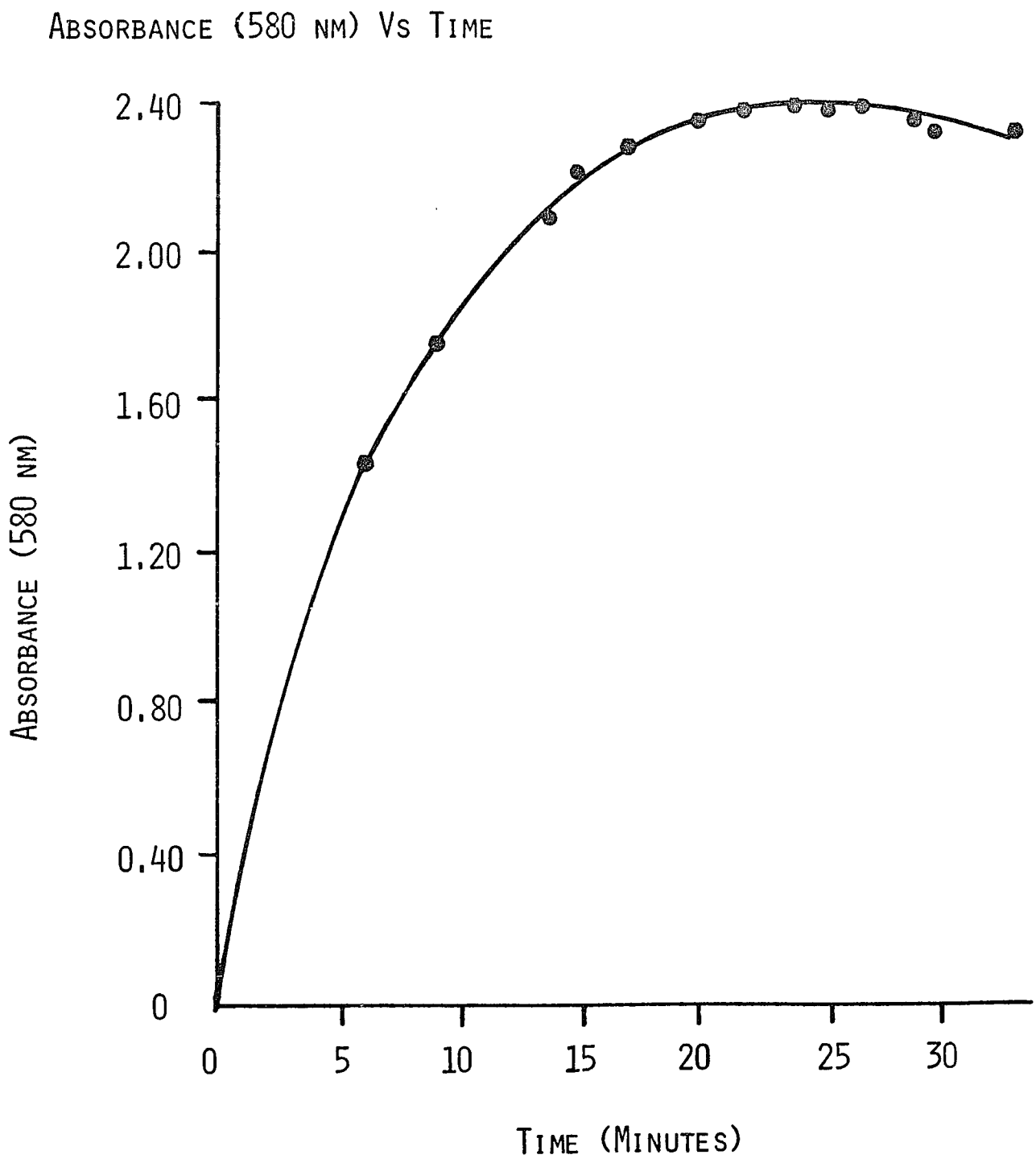
Color Development

The absorbance of the pararosaniline-formaldehyde adduct solution changes with time (Figure X), and the rate of change is a function of temperature (see below). When samples of formaldehyde were determined at 20-25°C, the maximum absorbance was attained from 22 to 28 minutes after the addition of the pararosaniline reagent. All determinations were carried out within this time range to ensure reproducibility.

Temperature Effects

As found by Saltzman,[79] an increase in temperature decreases the time required to reach maximum absorbance, although it also increases the rate of fading and decreases the time during which the absorbance is at a maximum; Saltzman found that the best temperature for the determination is between 20 and 25°C. Experiments carried out a temperature outside these limits should be standardized at that temperature, and the time and duration of maximum absorbance should be

FIGURE X


ABSORBANCE (580 NM) VS TIME
2.40
2.00
1.60
1.20
0.80
0.40
0
ABSORBANCE (580 NM)
0
5
10
15
20
25
30
TIME (MINUTES)

carefully determined.

STANDARDIZATION OF THE IODOMETRIC METHOD

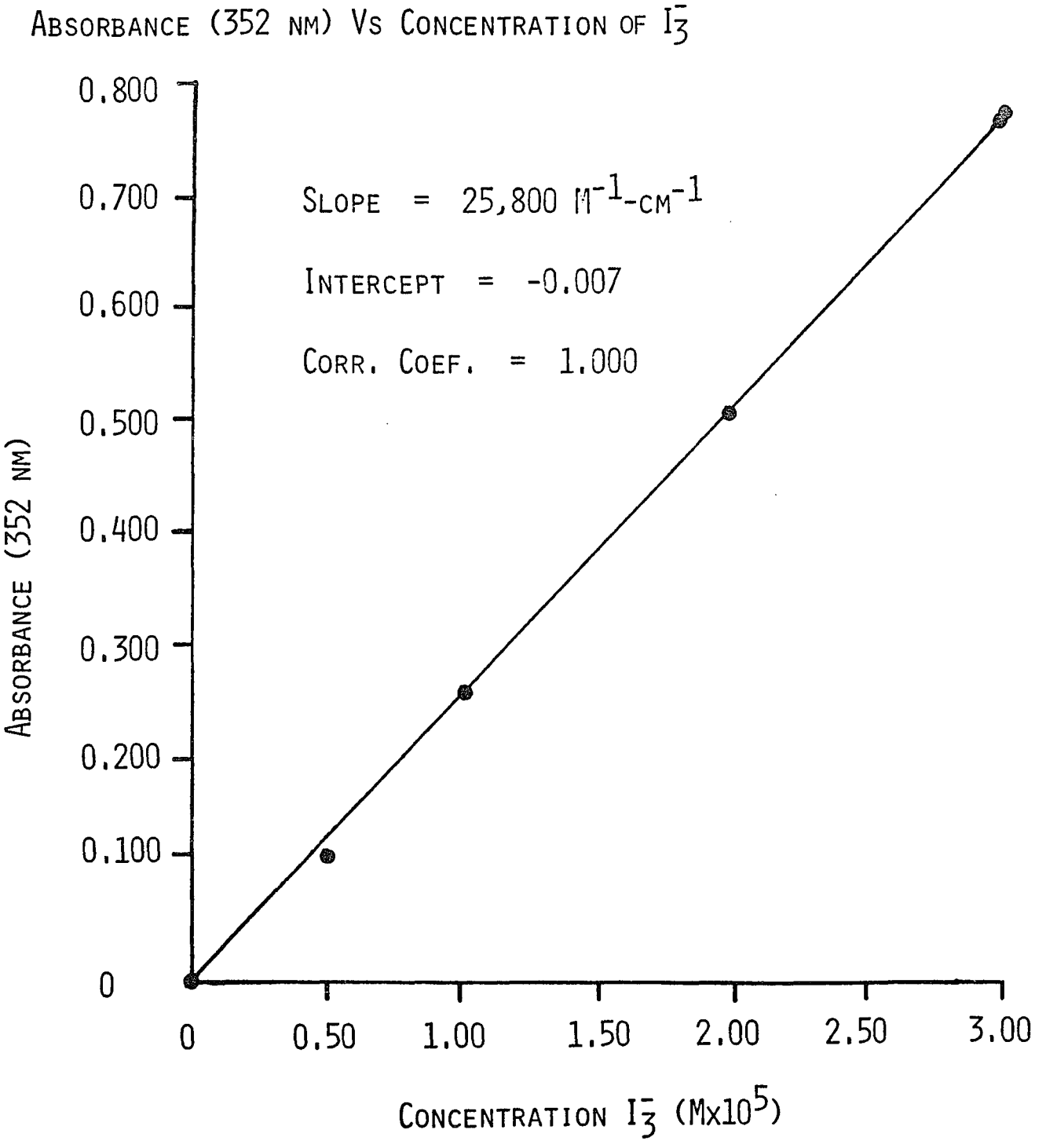
When the iodometric method was standardized according to the procedure outlined (see Experimental Section), a linear plot was obtained giving an extinction coefficient of 25,800 $\pm$ 1,300 M^{-1}-cm^{-1} (Figure XI). When standardization was repeated with fresh solutions prepared from the same reagents, an extinction coefficient of 23,850 $\pm$ 1,000 M^{-1}-cm^{-1} was obtained. The extinction coefficient of a given solution remained constant, within 6%, for 6 weeks, at which time they were discarded.

THE DETERMINATION OF OZONE BY OZONOLYSIS OF A TERMINAL OLEFIN

Solvent Study

Since several of the olefins tested as possible substrates for the ozone determination were solids, it was necessary to find a solvent which could be used in the olefin-ozone system, i.e., one which would dissolve the olefin, which would be sufficiently nonvolatile to remain in the impinger, and one which would not react with ozone. Three solvents: n-hexanol, hexadecane, and xylene were studied. Of these, n-hexanol gave positive blanks, and was unsatisfactory. Hexadecane is both nonvolatile and unreactive; however, the styrenoid molecules used as substrates were not sufficiently soluble in hexadecane. Xylene, a mixture of ortho and para, was best suited to the

FIGURE XI


Absorbance (352 nm) Vs Concentration of I_3^-
Slope = 25,800 M^{-1}-cm^{-1}
Intercept = -0.007
Corr. Coef. = 1.000
Absorbance (352 nm)
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0
0
0.50
1.00
1.50
2.00
2.50
3.00
Concentration I_3^- (Mx10^5)

olefin-ozone system; it is nonvolatile, unreactive, and almost completely miscible with the styrenoid molecules.

Olefin Study

Several styrenoid molecules were investigated in order to determine which gave the highest yield of formaldehyde. Vinyl anisole (4-methoxystyrene), eugenol (4-allyl-2-methoxyphenol), 2-vinylnaphthalene, and 1,1-diphenylethylene (DPE) were tested (Table II), and of these DPE gave the greatest yield of formaldehyde.

TABLE II

Olefin	Percent (in solvent)	CH_2O Yield ($\frac{\text{moles } CH_2O}{\text{mole } O_3}$)
vinyl anisole	1.0 (hexadecane)	0.23
eugenol	1.0 (hexadecane)	0.22
eugenol	neat[b]	0.67
vinyl naphthalene	0.1 (hexadecane)	0.26
vinyl naphthalene	0.5 (hexadecane)	0.29
vinyl naphthalene	1.0 (hexadecane)	0.52
vinyl naphthalene	10.0 (xylene)[c]	0.60
DPE	neat	0.90

[a]Uncorrected for trapping efficiency.
[b]West et al.[63]
[c]Highest convenient concentration for this solid olefin.

Stoichiometry Study

The yield of formaldehyde from the DPE determination method (see Experimental Section) was determined for a range of ozone concentrations (Table III). The flow rate used was 500 ml/minute.

TABLE III

Ozone (μg/g)	CH_2O Yield (±0.02)
0.09	0.922
0.24	0.914
0.43	0.908
0.88	0.877
1.91	0.924
1.94	0.898
3.31	0.899
4.02	0.867
5.11	0.899
5.20	0.886
6.37	0.785
7.76	0.765
8.70	0.750
9.20	0.708

Each of the reported yields is an average of at least three determinations of both ozone (by the iodometric method) and formaldehyde (by the pararosaniline method). The data below 5.20 μg/g inclusive give

an average yield of 0.90 ± 0.02 (Figure XII). Using a flow rate of 250 ml/minute, 8.40 μg/g ozone gave an 0.892 yield of formaldehyde, indicating that the losses at high levels of ozone with a flow rate of 500 ml/minute were due to incomplete trapping.

Interferences

The formaldehyde concentration in polluted air ranges between 0.005-0.15 μg/g,[80] thus formaldehyde interferes with the DPE determination method when ambient air samples are determined. However, the formaldehyde interference can be accounted for by the blank determination that is run with each set of determinations, and the interference only prevents the use of the method when formaldehyde concentrations are high and ozone concentrations are low.

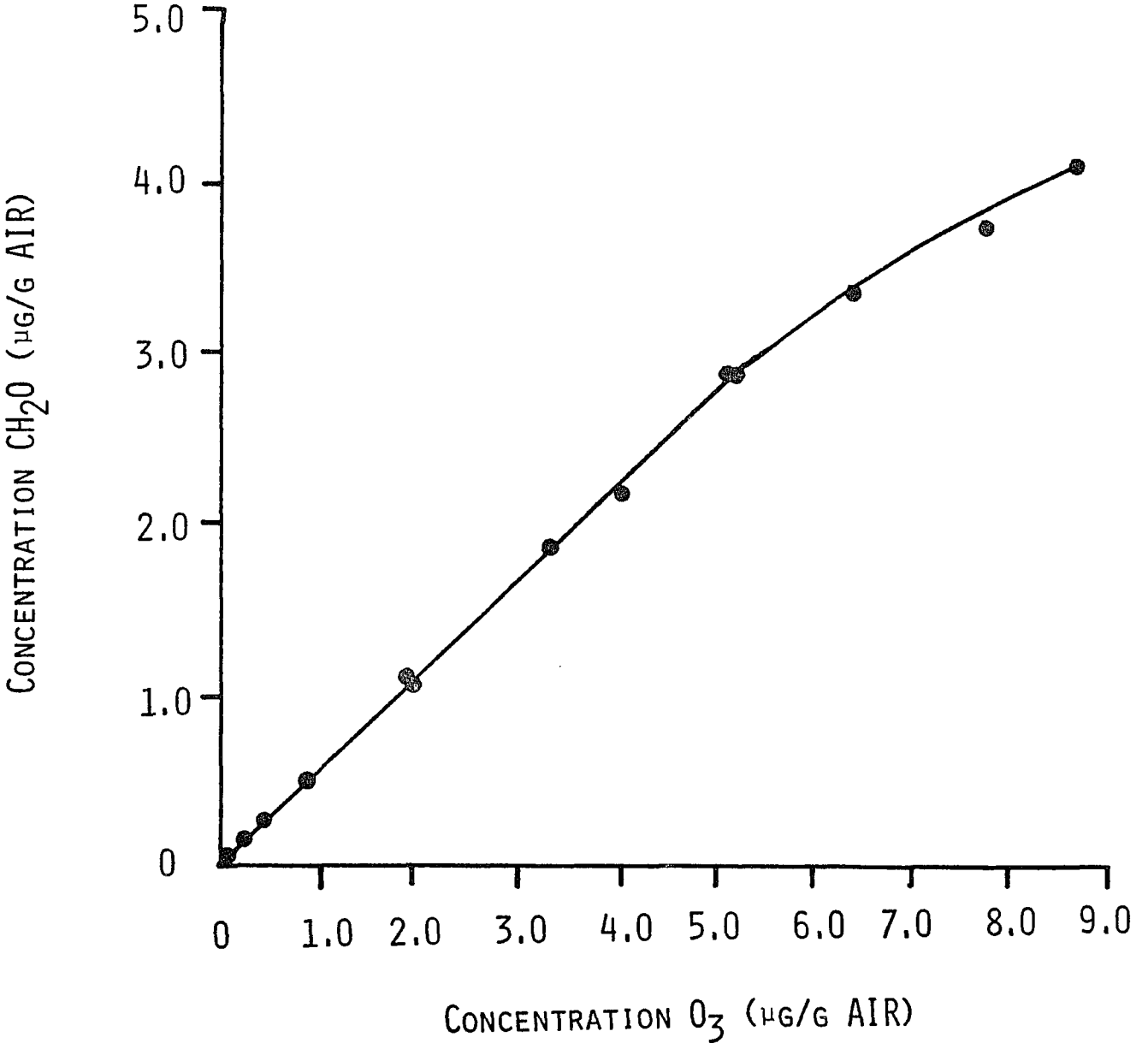
Although Hauser and Bradley[81] found that free radical initiators interfered with their 1,2-di-(4-pyridyl)ethylene ozone determination, West did not find any interference with his eugenol method.[63] As discussed in the introduction, this lack of interference in the eugenol method is probably due to the presence of the phenolic group in eugenol.[66] This hypothesis is supported by the finding that NO_2 interferes with the DPE method, when only neat DPE is used as the trapping agent. The yield of formaldehyde from 0.2 μg/g NO_2 was 2.50:1.00 formaldehyde: NO_2.

Inhibitor Study

Since the NO_2 interference was suspected to occur via a free radical initiated oxidation,[60] the effect of addition of a radical inhibitor was determined. Mesitol (2,4,6-trimethylphenol) was added

FIGURE XII

CONCENTRATION CH_2O VS CONCENTRATION OF O_3



as a solution in xylene. It was determined that levels of mesitol as high as 20% did not interfere with the determination of ozone by the DPE method. Levels of 1% and 2% were found to prevent the interference by NO_2 at levels up to 2 and 5 μg/g, respectively, and the addition of 2% mesitol was incorporated into the DPE determination procedure.

Conclusion

The DPE method is capable of determining ozone in the range 0.05-5.00 μg/g in the presence of up to 5 μg/g NO_2. Through this range the stoichiometry is 0.90±0.02:1.00 formaldehyde:ozone. A formaldehyde blank must be subtracted from each determination to account for solvent blank and for formaldehyde in the air being determined. The method is superior to the iodometric method in several ways. It has consistent stoichiometry which is not a function of many of the parameters to which the iodometric method is subject, it is not subject to interference from other oxidants in air, and it is, of course, free from interference by SO_2, which is one of the test reagents. While not as accurate or sensitive as the UV photometric method approved by the EPA,[16] the DPE method can be used for ozone determination in the presence of several species which interfere with the UV photometric method. Also the method does not require special equipment as do the UV photometric and the ethylene chemiluminescence methods. The DPE method is more sensitive than the West eugenol method[63] giving a 34% increase in sensitivity. Although best suited to laboratory determinations in the range 0.05-5.00 μg/g ozone, the DPE method is also satisfactory for use in ozone determinations in

polluted air, and is superior to the iodometric method for such determinations.

PART I

REFERENCES

1. N. Kornblum, R. A. Smiley, R. K. Blackwood, and D. C. Iffland, "The Mechanism of the Reaction of Silver Nitrite with Alkyl Halides. The Contrasting Reactions of Silver and Alkali Metal Salts with Alkyl Halides. The Alkylation of Ambident Anions." J. Am. Chem. Soc., 77, 6269 (1955).

2. J. Kagawa and T. Toyama, "Photochemical Air Pollution: It's Effects on Respiratory Function of Elementary School Children." Arch. Environ. Health, 30, 117 (1975).

3. R. A. Shakman, "Nutritional Influences on the Toxicity of Environmental Pollutants." Arch. Environ. Health, 28, 105 (1974).

4. "Air Quality Criteria for Photochemical Oxidants." U.S. Dept. HEW National Air Pollution Control Administration Publ. No. AP-63, Wash., (1970).

5. J. N. Pitts, Jr. and R. L. Metcalf, Editors, "Advances in Environmental Science and Technology," Wiley-Interscience, N.Y., N.Y. (1969).

6. W. A. Pryor, J. P. Stanley, E. Blair, and G. B. Cullen, " Autoxidation of Polyunsaturated Fatty Acids: Part I. Effects of Ozone on the Autoxidation of Neat Methyl Linoleate and Methyl Linoleate." Arch. Environ. Health, 31, 201 (1976).

7. S. L. Sachdev, J. P. Lodge, and P. W. West, "Specific Method for the Determination of Ozone in the Atmosphere." Anal. Chim. Acta, 58, 141 (1972).

8. P. L. Asquith, "The Stability of Gaseous Nitrous Acid." Chem. Comm., 1970, 744 (1970).

9. John W. Lightsey, Dissertation in Preparation, Louisiana State Univ.

10. L. Reich and S. S. Stivala, "Autoxidation of Hydrocarbons and Polyolefins: Kinetics and Mechanism." Marcel Dekker, Inc., New York, N.Y., (1969), p. 31.

11. W. A. Pryor, "Free Radicals." McGraw-Hill, New York, N.Y., (1966), p. 231.

12. S. Siggia, "Quantitative Organic Analysis via Functional Groups," 3rd ed., Wiley, New York, N.Y., (1963), p. 526.

13. C. Ellis and A. Vogel, "Studies on Hypovanadous Salts as Analytical Reagents." Analyst, 81, 693 (1956).

14. H. Juker, "Zur Direkten Titration Organischer Verbindungen mit Chrom (II) Sulfat." Anal. Chim. Acta, 16, 210 (1957).

15. L. Jones and J. Riddick, "Volumetric Determination of Primary and Secondary Nitroparaffins." Anal. Chem., 28, 1137 (1956).

16. P. C. Gangwar, J. P. Sharma, and R. D. Tiwari, "Semimicrodetermination of Nitro Groups by Reduction with $SnCl_2$." Proc. Nat. Acad. Sci., India, Sec. A., Vol. 32, Part II, (1962), p. 119.

17. J. P. Tandon, "Studies in Bivalent Chromium Salts: Part VIII. Estimation of Nitro, Nitroso, and Azo Compounds, Quinones and Carbohydrates with Chromous Sulphate." Z. Anal. Chem., 167, 184 (1959).

18. T. S. Ma and J. V. Early, "Titanous Chloride as a Reagent for Quantitative Organic Microanalysis." Mikrochim. Acta, 1, 129 (1959).

19. T. DeVries and R. Ivett, "A Polographic Study of Aliphatic Nitro Compounds." Ind. Eng. Chem., Anal. Ed., 13, 339 (1941).

20. M. Matsui and H. Sago, "Controlled-Potential Electrolysis: Part VI. Reductions of 2,2-Dinitropropane." J. Chem. Soc., 4773 (1961).

21. V. B. Ehlers and J. W. Sease, "Constant-Potential Coulometric Reductions of Organic Nitro and Halogen Compounds." Anal. Chem., 31, 16 (1959).

22. "Standard Methods for the Examination of Water and Waste Water, Natural and Treated Waters (100)." 13th ed., Amer. Pub. Health Assoc., N.Y., N.Y., (1971), p. 246.

23. "Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties," No. 9 Agronomy Series, Amer. Soc. of Agronomy, Inc., Mad., Wisc., (1965), p. 1151.

24. Y. A. Gawargious, "The Determination of Nitro and Related Functions," Academic Press, N.Y., N.Y., (1973), p. 8.

25. F. Turba, R. Haul, and G. Uhlen, "Kolorimetrische Bestimmung von Nitroparaffinen." Angew. Chem., 61, 74 (1949).

26. A. P. Altshuller and I. R. Cohen, "Determination of Primary Nitroparaffins by the Nitrous Acid Reaction." Anal. Chem., 32, 881 (1960).

27. F. Feigl and V. Gentil, "Spot Reaction for Acidic Polynitro Compounds." Anal. Chem., 27, 432 (1955).

28. L. Jones and J. Riddick, "Colorimetric Determination of 2-Nitro-1,1-bis(p-chlorophenyl) alkanes." Anal. Chem., 23, 349 (1951).

29. E. Scott and J. Treon, "Colorimetric Determination of Primary Mononitroparaffins." Eng. Chem., Anal. Ed., 12, 189 (1940).

30. A. Gordon and R. Ford, "The Chemist's Companion." Wiley-Interscience, N.Y., N.Y., (1972), p.52.

31. K. G. Stone, "Determination of Organic Compounds." McGraw-Hill, N.Y., N.Y., (1956).

32. K. Grebber and J. V. Karabinos, "A Study of the Diphenylanine Test for Aliphatic Nitrocompounds." J. Research Natl. Bur. Standards, 49, 163 (1952).

33. M. R. F. Ashworth and E. Gramsch, "Colorimetric Determination of Nitroalkane via the Janovsky Reaction." Mikrochim. Acta, 2, 358 (1967).

34. L. Legradi, "Nachweis von Nitroverbindungen." Mikrochim. Acta, 4, 739 (1968).

35. G. Talsky, "Verwendung von Aluminiumchlorid zur Gruppenanalyse Organischer Verbindungen. I. Aliphsren und Cycloaliphsten." Z. Anal. Chem., 188, 417 (1962).

36. E. Trachman, A. Fono, and T. S. Ma, "Micro Analysis of Nitro Compounds: III. Detection of Nitro Compounds on the Thin-Layer Chromplate." Mikrochim. Acta, 6, 1185 (1968).

37. C. Paleari and F. Renzanigo, "Analysis of Engine Exhaust Gas." Riv. Combustibili, 12, 966 (1958).

38. R. B. L. Gwatkin, "Estimation of Aliphatic Nitro Compounds." Nature, 193, 973 (1962).

39. L. Jones and J. Riddick, "Colorimetric Determination of Nitroparaffins." Anal. Chem., 24, 1533 (1952).

40. P. K. Bose, "A New Method for the Detection of the Nitro-Group in Some Organic Compounds." Analyst, 56, 504 (1931).

41. R. L. Sweet, R. S. Spindt, and V. D. Meyer, "A New Method for the Determination of Aliphatic Nitro Compounds." Am. Chem. Soc. Div. of Pet. Chem., Gen. Papers, 33 (1955).

42. Griess and Ilosvay, Bull. Soc. Chim., 2, iii, 388 (1899).

43. R. S. Weston, "Notes on the Determination of Nitrogen as Nitrites in Waters." J. Am. Chem. Soc., 27, 281 (1905).

44. T. Meisel and L. Erdey, "Empfindliche Reaktion zum Nachweis Aliphatischer Nitroverbindungon." Mikrochim. Acta, 6, 1148 (1966).

45. F. L. Estes and P. K. Baughman, "Determination of 2-Nitropropane and of Nitrite in Mixtures." Anal. Chem., 33, 473 (1961).

46. K. Johnson and E. F. Degering, "The Utilization of Aliphatic Nitro Compounds (I) The Production of Amines and (II) The Production of Oximes." J. Am. Chem. Soc., 61, 3194 (1939).

47. P. Aeberli and W. J. Houlihan, "The Selective Reduction of a Nitro and Pyridyl Group." J. Org. Chem., 32, 3211 (1967).

48. J. F. Knifton, "Homogeneous Catalyzed Reduction of Nitro-Compounds. III. Synthesis of Aliphatic Amines." J. Org. Chem., 40, 519 (1975).

49. N. Kornblum and L. Fishbein, "The Reduction of Optically Active 2-Nitrooctane and α-Phenylnitroethane." J. Am. Chem. Soc., 77, 6266 (1955).

50. R. F. Nystrom and W. G. Bron, "Reduction of Organic Compounds by Lithium Aluminum Hydride III. Halides, Quinones, Miscellaneous Nitrogen Compounds." J. Am. Chem. Soc., 70, 3738 (1948).

51. H. C. Brown, P. M. Weissman, and N. M. Yoon, "Selective Reductions IX. Reaction of Lithium Aluminum Hydride with Selected Organic Compounds Containing Representative Functional Groups." J. Am. Chem. Soc., 88, 1458 (1966).

52. H. Reinheckel and G. Tauber, "Anomalies from the Reduction of α-Nitro Fatty Acid Esters." Monatsber. deut. Akad. Wiss. Berlin, 1, 43 (1959).

53. W. T. Smith, Jr. and J. M. Patterson, "Functional Group Analysis." Anal. Chem., 44, 207R (1972).

54. H. S. Nitzsche and M. Wick, "Reduktionen mit Methyl-wasserstoff-Polysiloxanen: Verfahren zur Reduktion Organischer Verbindungen Besonders in Nicht-Wassriger Lösung." Angew. Chem., 69, 96 (1957).

55. J. Lipowitz and S. A. Bowman, "Use of Polymethylhydrosiloxane as a Selective, Neutral Reducing Agent for Aldehydes, Ketones, Olefins, and Aromatic Nitro Compounds." J. Org. Chem., 38, 162 (1973).

56. J. Lipowitz and S. A. Bowman, "Reducing Agent-Polymethylhydrosiloxane (PMHS) Carbocation and Onium Ion Reagents." Aldrichimica Acta, 6, 1 (1973).

57. H. J. Klimisch and L. Stadler, "Mikroquantitative Bestmmung von Aliphatischen Aminen mit 7-Chloro-4-Nitrobenzo-2-Oxa-1,3,-Diazol." J. Chromatogr., 90, 141 (1974).

58. F. C. McIntire, L. M. Clements, and M. Sproull, "1-Fluoro-2,4-dinitrobenzene as a Quantitative Reagent for Primary and Secondary Amines." Anal. Chem., 25, 1757 (1953).

59. N. K. Mathur, "Newer Acylation Methods for the Determination of Organic Hydroxyl and Amino Compounds." Talanta, 13, 1601 (1966).

60. J. H. Ross, "2,6,-Dichloroquinone 4-Chloroimide as a Reagent for Amines and Aromatic Hydrocarbons on Thin-Layer Chromatograms." Anal. Chem., 40, 2138 (1968).

61. S. I. Obtemperanskaya, A. P. Terent'ev, Yu. N. Tikhonov, I. Ya. Tsyokina, "Spectrophotometric Determination of Primary and Secondary Amines using 2,4-Dinitroflourobenzene." Chem. Abstr., 80, 108920e (1974).

62. L. S. Chemodanova, "Determination of Toxic Substances in Preparation of High-Molecular Weight Amines." Chem. Abstr., 71, 128312m (1969).

63. J. T. Stewart and D. M. Lotti, "Improved Colorimetric Determination of Primary Aromatic Amines with 9-Chloroacridine: Application to Some Local Anesthetics." J. Pharm. Sci., 59, 838 (1970).

64. G. R. Umbreit and R. L. Houtman, "Colorimetric Analysis of Organic Hydroxyl Groups." J. Pharm. Sci., 56, 349 (1967).

65. S. N. Chakravarti and M. B. Roy, "A New Colorimetric Test for Novocaine and Primary Amines." Analyst, 62, 603 (1937).

66. S. I. Obtemperanskaya and V. N. Likhosherstova, "Spectrophotometric Determination of Primary and Secondary Amines Using 3,5-Dinitrolbenzoyl Chloride." Chem. Abstr., 80, 108919m (1974).

67. S. I. Obtemperanskaya, V. N. Likhosherstova, and A. P. Terent'ev, "2,4-Dinitrobenzoyl Chloride as a New Reagent for the Spectrophotometric Determination of Aromatic, Aliphatic, and Alicyclic Amines." Zh. Anal. Khim., 25, 2033 (1970).

68. J. Bartos and J. F. Butrin, "Use of Quinazolinium Salts in Analysis of Primary Amines." Ann. Pharm. Frac., 17, 144 (1959).

69. M. J. Kolbezen, J. W. Eckert, and B. F. Bretschnieder, "The Microdetermination of Amines with 2,4-Dinitroflourobenzene." Anal. Chem., 34, 583 (1962).

70. M. Richardson, "Spectrophotometric Micro-Estimation of Some Simple Plant Amines." Nature, 197, 290 (1963).

71. W. E. Mathewson, "Reagents for the Detection and Estimation of Aliphatic Amines and Nitro Paraffins." Trans. Kansas Acad. Sci., 58, 282 (1955).

72. F. Critchfield and J. Johnson, "Colorimetric Determination of Primary Aliphatic Amines by Copper Salicylaldehyde Method." Anal. Chem., 28, 436 (1956).

73. A. J. Milun, "Colorimetric Determination of Primary Amine in Fatty Amino Acetates and Fatty Amines." Anal. Chem., 29, 1502 (1957).

74. T. Tsukamoto and K. Yuhi, "Halosalicylaldehyde: II. Spectrophotometric Determination of Primary Amines by 3,5-Dibromosalicylaldehyde." Chem. Abstr., 54, 4476e (1960).

75. T. Tsukamoto and K. Yuhi, "Halosalicylaldehydes: IV. Spectrophotometric Determination of Amino Acids by 3,5-Dibromosalicylaldehyde." Chem. Abstr., 55, 13327d (1961).

76. T. Tsukamoto and K. Yuhi, "Halosalicylaldehyde: III. Colorimetric Determination of Aliphatic Primary Amines by 3,5-Dibromosalicylaldehyde." Chem. Abstr., 54, 20962e (1960).

77. J. L. Valentine, J. B. Entrikin, and M. W. Hanson, "A Test for the Subclasses of Aliphatic Amines." J. Chem. Educ., 41, 569 (1964).

78. H. M. Hershenson and D. N. Hume, "Photometric Determination of Aliphatic Amines." Anal. Chem., 29, 16 (1957).

79. M. Yamagishi, "Ninhydrin Reaction: VIII. Ninhydrin of $RR'CHNH_2$-Type Compounds and its Homologs." Chem. Abstr., 49, 3736e (1955).

80. P. J. Lloyd and A. D. Carr, "The Determination of Small Amounts of Amine in Aqueous Solution." Analyst, 86, 335 (1961).

81. S. Ohduma and Y. Kido, "Detection of Weak Amines: III. Methylephedrine." Chem. Abstr., 51, 7950i (1957).

82. F. E. Brown and J. Wolt, "Identification of Amines." Proc. Louisiana Acad. Sci., 18, 95 (1955).

83. J. Bartos and M. Pesez, "No. 282 - Colorimetrie des Alcoylamines Primaires et Secondaires et des Arylamines Primaires a l'aide de l'acide Naphroquinone-1,2 sulfonique-4, sensibilisation de la methode." Bull. Soc. Chim. Fr., 4, 1627 (1970).

84. R. M. Silverstein, "Spectrophotometric Determination of Primary, Secondary, and Tertiary Fatty Amines in Aqueous Solution." Anal. Chem., 35, 154 (1963).

85. N. H. Irving and J. J. Markham, "The Determination of Small Amounts of Long-Chain Tertiary Alkylamines and Quarternary Ammonium Salts." Anal. Chim. Acta, 39, 7 (1967).

86. A. J. Milun, "Determination of Small Amounts of Secondary Amine in High Molecular Weight Fatty Primary Amines." Anal. Chem., 31, 1655 (1959).

87. S. Underfriend, S. Stein, P. Boehlen, W. Dairman, W. Leimgruber, and M. Weigele, "Fluorescamine: A Reagent for Assay of Amino Acids, Peptides, Protions, and Primary Amines in the Picomole Range." Science, 178, 871 (1972).

88. B. M. C. Hopewell and J. E. Page, "Colorimetric Estimation of Long-Chain Aliphatic Amines." Analyst, 70, 17 (1945).

89. M. Pesez and J. Bartos, "Colorimetry of Alkylamines and Nitrogenous Heterocyclic Compounds by the Formation of Intermediate o-Quinones." Ann. Pharm. Franc., 23, 781 (1965).

90. J. Bartos, "No. 57 - Colorimetrie des Amines Aliphatiques Secondaires a l'aide de la Dicyano-2,3 Dichloro-5,6 Benzoquinone (Note de Laboratoire)." Bull. Soc. Chim. France, 1, 331 (1966).

91. V. P. Zhivopistsev, E. A. Selezneva, Z. I. Bragina, and A. P. Lipchina, "Photometric Determination of Small Amounts of Aliphatic Amines with Bis(4-dimethylaminophenyl)-[1-(p-sulfo-phenyl)-3-methylpyrazolon-5-yl] Methanol." Chem. Abstr., 70, 25499c (1969).

92. R. M. Silverstein, "Colorimetric Determination of Primary and Secondary Amines." Am. Chem. Soc., Div. Water Waste Chem., Preprints, March, 15 (1962).

93. I. M. Korenman and N. A. Shemarova, "Extraction-Photometric Determination of Low Molecular Weight Aliphatic Amines." Chem. Abstr., 82, 51126w (1975).

94. A. Mukerjee and P. Mukerjee, "Spectrophotometric Analysis of Long-Chain Amines by a Dye-Extraction Method." Chem. Abstr., 57, 4039c (1962).

95. N. Kornblum, "Synthesis of Aliphatic Nitro Compounds." J. Org. Chem., 22, 455 (1957).

96. J. Zavada, M. Svoboda, and M. Pakova, "The Role of Base Ion Pairing in Bimolecular Elimination: The Effect on the cis-to-trans-olefin Ratios in the Anti- and the Syn-Pathway." Td. Lett., 711 (1972).

97. S. L. Regen, "Triphase Catalysis." J. Amer. Chem. Soc., 97, 5956 (1975).

98. Vogel, "Practical Organic Chemistry," 3rd Ed., Longman Group, Ltd., (1956), p. 825.

99. W. K. Seifert, "Synthesis of Nitro-olefins from Olefin Dinitrogen Tetroxide Adducts." J. Org. Chem., 52, 125 (1963).

100. R. M. Silverstein, G. C. Bassler, and T. C. Morrill, "Spectrometric Identification of Organic Compounds." 3rd Ed., Wiley-Interscience, N.Y., N.Y., (1974), p. 213.

101. M. J. Astle and F. P. Abbott, "Catalysis by Anion Exchange Resins; Condensation of Nitroparaffins with Aldehydes and Ketones." J. Org. Chem., 21, 1228 (1956).

102. H. E. Baumgarten, "Organic Synthesis, Collected Volume 5." Wiley Inc., N.Y., N.Y., (1973), p. 833.

103. C. A. Streuli and P. R. Averell, "Chemical Analysis, Vol. 28, The Analytical Chemistry of Nitrogen and Its Compounds." Wiley-Interscience, N.Y., N.Y., (1970), p. 446.

104. G. Grady, Dept. of Chemistry, St. Michael's College, Winooski, Vt., Personal Communication.

105. N. Kornblum and J. W. Powers, "Synthesis of Aliphatic Nitro Compounds," J. Org. Chem., 22, 455 (1957).

106. N. Kornblum, H. O. Larsen, R. K. Blackwood, D. D. Mooberry, E. P. Oliveto, and G. E. Graham, "A New Method for the Synthesis of Aliphatic Nitro Compounds." J. Am. Chem. Soc., 78, 1497 (1956).

107. A. C. Cope, Ed., "Synthesis of Aliphatic and Alicyclic Nitro Compounds." Org. Reactions, 12, 114 (1962).

PART II

REFERENCES

1. H. E. Stokinger, "Ozone Toxicology." Arch. Env. Health, 10, 719 (1965).

2. C. M. Birdsall, A. C. Jenkins, and E. Spadinger, "Iodometric Determination of Ozone." Anal. Chem., 24, 662 (1952).

3. M. L. Kilpatrick, C. C. Herrick, and M. Kilpatrick, "The Decomposition of Ozone in Aqueous Solution," J. Am. Chem. Soc., 78, 1784 (1956).

4. G. R. Burguieres, Master's Thesis, Louisiana State University, Baton Rouge, Louisiana, Dec. 1978.

5. L. R. B. Yeatts, and H. Taube, "The Kinetics of the Reaction of Ozone and Chloride Ion in Acid Aqueous Solution." J. Am. Chem. Soc., 71, 4100 (1949).

6. W. A. Pryor and M. E. Kurz, "Radical Production from the Interaction of Closed Shell Molecules. VIII The Mechanism of the Reaction of Ozone with t-Butyl Hydroperoxide." Td. Lett., In Press.

7. M. G. Alder and G. R. Hill, "The Kinetics and Mechanism of Hydroxide Ion Catalyzed Ozone Decomposition in Aqueous Solution," J. Am. Chem. Soc., 72, 1884 (1950).

8. G. Slomp, Jr., "Ozonization of Methylene Chloride and Chloroform " J. Org. Chem., 22, 1277 (1957).

9. T. W. Nakagawa, L. J. Andrews, and R. M. Keefer, "The Kinetics of Ozonization of Polyalkylbenzenes." J. Am. Chem. Soc., 82, 269 (1960).

10. D. G. Williamson and R. J. Cvetanovic, "Rates of Ozone-Paraffin Reactions in Carbon Terrachloride Solution." J. Am. Chem. Soc., 92, 2949 (1970).

11. S. Miyano, M. Hida, and H. Hashimoto, "Reaction of Benzaldehyde with Zinc-Polyhalomethane Complexes Obtained from Polyhalomethanes and Zinc-Copper Couple." J. Organmet. Chem., 12, 263 (1968).

12. C. Brown and K. D. Franson, "Manometric Determination of the Density of Liquid Ozone." J. Chem. Phys., 21, 917 (1953).

13. M. Griggs, "Abosrption Coefficients of Ozone in the Ultraviolet and Visible Regions." J. Chem. Phys., 49, 857 (1968).

14. W. B. Demore and O. Raper, "Hartley Band Extinction Coefficients of Ozone in the Gas Phase and in Liquid Nitrogen, Carbon Monoxide, and Argon." J. Phys. Chem., 68, 412 (1964).

15. P. L. Harst, E. A. Stephens, W. E. Scott, and R. C. Doerr, "Absorptivities for the Infrared Determination of Trace Amounts of Ozone." Anal. Chem., 33, 1113 (1961).

16. "Photochemical Oxidants; Measurement of Ozone in the Atmosphere; Requirements for Preparation, Adoption, Submittal of Implementation Plans." Fed. Regis., 43, 26971 (1978).

17. "Appendix D. Reference Method for the Measurement of Photochemical Oxidants Corrected for Interferences Due to Nitrogen Oxides and Sulfur Oxides." Fed. Regis., 36 (228), 22392 (Nov. 25, 1971).

18. N. Allen, "Quantitative Determination of Small Amounts of Hydrogen Peroxide and of Ozone." Ind. Eng. Chem. Anal. Ed., 2, 55 (1930).

19. D. L. Flamm and S. A. Anderson, "Iodate Formation and Decomposition in Iodometric Analysis of Ozone," Env. Sci. and Tech., 9, 660 (1975).

20. D. L. Flamm, "Analysis of Ozone at Low Concentrations with Boric Acid Buffered KI." Env. Sci. and Tech., 11, 978 (1977).

21. D. H. Byers and B. E. Saltzman, "Determination of Ozone in Air by Neutral and Alkaline Iodide Procedures." Adv. Chem. Series, 21, 93 (1959).

22. B. E. Saltzman and N. Gilbert, "Iodometric Determination of Organic Oxidants and Ozone." Anal. Chem., 31, 1914-20 (1959).

23. E. D. Boelter, G. L. Putnam, and E. I. Lash, "Iodometric Determination of Ozone of High Concentration." Anal. Chem., 22, 1533 (1950).

24. "Air Quality Criteria for Photochemical Oxidations." USD HEW PHS, Nat. Air Pollut. Contr. Admin., Publ. No. AP-63, Washington, D. C., March (1970).

25. A. W. Boyd, C. Willis, and R. Cyr, "New Determination of Stoichiometry of the Iodometric Method for Ozone Analysis at pH 7.0." Anal. Chem., 42, 670 (1970).

26. S. L. Kopczynski and J. J. Bufalini, "Some Observations on Stoichiometry of Iodometric Analyses of Ozone at pH 7.0." Anal. Chem., 43, 1126 (1971).

27. R. S. Ingols, R. H. Fether, and W. E. Eberhardt, "Determining Ozone in Solution." Adv. Chem. Ser., 21, 102 (1959).

28. J. A. Hodgeson, R. E. Baumgardner, B. E. Martin, and K. A. Rehme, "Stoichiometry in the Neutral Iodometric Procedure for Ozone by Gas-Phase Titration with Nitric Oxide." Anal. Chem., 43, 1123 (1971).

29. R. N. Dierz, J. Pruzansky, and J. D. Smith, "Effect of pH on the Stoichiometry of the Iodometric Determination of Ozone." Anal. Chem., 45, 402 (1973).

30. L. R. Schmitz, "Stoichiometry of Ozone-Iodide Reaction: Significance of Iodate Formation." Env. Sci. and Tech., 7, 647 (1973).

31. E. P. Parry, "Stoichiometry of Ozone-Iodide Reaction: Significance of Iodate Formation." Env. Sci. and Tech., 7, 647 (1973).

32. B. E. Saltzman, "Determination of Oxidants (Including Ozone): Neutral Buffered-Potassium Iodide Method." Env. Health Ser. Air Poll., Selected Methods for the Measurement of Air Pollutants, May (1965).

33. B. E. Saltzman, "Colorimetric Microdetermination of Nitrogen Dioxide in the Atmosphere." Anal. Chem., 26, 1949 (1954).

34. I. R. Cohen, T. C. Purcell, and A. P. Alshuller, "Analysis of the Oxidant in Photooxidation Reactions." Env. Sci. and Tech., 1, 247 (1967).

35. J. P. Stanley, E. Blair, G. B. Cullen, and W. A. Pryor, "Autoxidation of Polyunsaturated Fatty Acids. Part I. Effect of Ozone on the Autoxidation of Neat Methyl Linoleate and Methyl Linoenite." Arch. Env. Health, 31, 201 (1976).

36. S. R. Goates, J. S. Bradshaw, and N. F. Mangelson, "Determination of Ozone in the 5-ppb to 100-ppb Range by a Modified Saltzman Technique." Anal. Chem., 47, 1690 (1975).

37. C. H. Li and C. F. White, "Kinetics of Hypiodite Decomposition." J. Am. Chem. Soc., 65, 335 (1943).

38. D. H. Stedman, E. E. Daby, F. Stuhl, and H. Niki, "Analysis of Ozone and Nitric Oxide by a Chemiluminescent Method in Laboratory and Atmospheric Studies of Photochemical Smog." J. Air. Poll. Assoc., 22, 260 (1972).

39. R. D. Martini, "Determination of Nitrogen Dioxide and Nitric Oxide in the Parts Per Million Range in Flowing Gaseous Mixtures by Means of the Nitrate-Specific-Ion Electrode." Anal. Chem., 42, 1102 (1972).

40. J. J. Bufalini, "Gas Phase Determination of Atmospheric Ozone." Env. Sci. and Tech., 2, 703 (1968).

41. A. F. Warsburg, A. W. Brewer, and J. P. Lodge, "Evaluation of a Coulometric Oxidant Sensor." Intern. J. Air Water Poll., 8, 21 (1964).

42. H. Watanabe and T. Nakadoi, "Fluorophotometric Determination of Trace Amounts of Atmospheric Ozone." J. Air. Poll. Contr. Assoc., 16, 614 (1966).

43. C. O. Hommel, D. Chelck, and F. J. Brousaides, "Ozone Analyzer Users Radioactive Clathrate." Nucleonics, 17, 94 (1959).

44. D. J. Chelck and C. A. Ziegler, "Krypton in a Case-Clathrate Beta Sources," Nucleonics, 17, 130 (1959).

45. C. E. Bradley and A. J. Haagen-Smit, "The Application of Rubber in the Quantitative Determination of Ozone." Rubber Chem. and Tech., 24, 750 (1951).

46. R. H. Hendricks and L. B. Larsen, "An Evaluation of Selected Methods of Collection and Analysis of Low Concentrations of Ozone." Am. Ind. Hyg. Assoc. J., 27, 80 (1966).

47. A. J. Haagen-Smit and A. J. Brunelle, "The Application of Pheonolphthalein Reagent to Atmospheric Oxidant Analysis." Intern. J. Air Poll., 1, 51 (1958).

48. H. H. Bovee and R. J. Robinson, "Sodium Diphenylamine as an Analytical Reagent for Ozone." Anal. Chem., 33, 1115 (1961).

49. M. S. Egorov, "A New Method for the Estimation of Ozone in Air." Z. Untersuch Lebenam, 56, 355 (1928).

50. R. K. Stevens and A. E. O'Keeffe, "Modern Aspects of Air Pollution Monitoring." Anal. Chem., 42, 143A (1970).

51. O. S. Privett and E. C. Nickell, "Stereoisomer Formation on the Ozonization of Esters of Monosaturated Fatty Acids." J. Lipid Res., 4, 208 (1963).

52. P. S. Bailey, "The Ozonolysis of Phenanthrene in Methanol." J. Am. Chem. Soc., 78, 3811C (1956).

53. W. P. Keaveney and J. J. Pappas, "Direction of Cleavage of Primary Ozonides from p-Substituted Propenylbenzenes." Td. Lett., 10, 841 (1969).

54. D. G. Williamson and R. J. Cvetanovic, "Rates of Ozone-Olefin Reactions in Carbon Tetrachloride Solutions." J. Am. Chem. Soc., 90, 3668 (1968).

55. W. S. Knowdes and Q. E. Thompson, "A Convenient Method for Reduction of Hydroperoxide Ozonation Products." J. Org. Chem., 25, 1031 (1960).

56. P. S. Bailey and S. S. Bath, "Ozonolysis of 1,2-Dibenzoylpropene and 1,2-Dibenzoylethylene. Markovnikoff's Rule and the Initial Attack of Ozone on an Unsaturated System." J. Am. Chem. Soc., 79, 3120 (1957).

57. C. S. Marvel and V. Nichols, "The Formation of Dimeric Peroxides by Ozonization of Olefins." J. Org. Chem., 6, 296 (1941).

58. H. A. Brave and J. P. Lodge, Jr., "Specific Spectrophotometric Determination of Ozone in the Atmosphere." Anal. Chem., 36, 671 (1964).

59. E. Sawicki, T. Stanley, and T. Hauser, "Detection of Aromatic Aldehydes." Chem. Analyst, 47, 31 (1958).

60. F. R. Mayo and A. A. Miller, "Oxidation of Unsaturated Compounds. I. The Oxidation of Syrene." J. Am. Chem. Soc., 78, 1017 (1956).

61. T. A. Hauser and D. W. Bradley, "Specific Spectrophometric Determination of Ozone in the Atmosphere Using 1,2-Di-(4-pyridyl)ethylene." Anal. Chem., 38, 1529 (1966).

62. T. R. Hauser and D. W. Bradley, "Effect of Interfering Substances and Prolonged Sampling on the 1,L-Di-(4-pyridyl)ethylene Method for Determination of Ozone in Air." Anal. Chem., 39, 1184 (1967).

63. P. W. West, S. L. Sachdev, and L. P. Lodge, Jr., "Specific Method for the Determination of Ozone in the Atmosphere." Anal. Chim. Acta, 58, 141 (1972).

64. G. R. Lyles, F. B. Dowling, and V. J. Blanchard, "Quantitative Determination of Formaldehyde in the Parts Per Hundred Million Concentration Level." J. Air Poll. Contr. Assoc., 15, 106 (1965).

65. J. Knights and E. S. Waight, "Observations on the Ozonolysis of Allylic Compounds. The Structure of Geraniol." J. Chem. Soc., 2830, (1955).

66. S. Fliszar and M. Granger, "A Quantitative Investigation of the Ozonolysis Reactions. VIII. On the Direction of Cleavage of Primary Ozonides of Selected Unsymmetrical Olefins." J. Am. Chem. Soc., 91, 3330 (1969).

67. R. H. Callighan and M. H. Wilr, "Ozonolysis of Vinylpyridines." J. Org. Chem., 26, 4912 (1961).

68. S. Fliszar, "Ozonolysis of Nucleus-Substituted Styrenes." Td. Lett., 49, 6083 (1966).

69. A. P. Altshuller, D. L. Miller, and S. F. Sleva, "Determination of Formaldehyde in Gas Mixtures by the Chromotropic Acid Method." Anal. Chem., 33, 621 (1961).

70. A. G. Smith (Chairman). "Tentative Method of Analysis for Formaldehyde Content of the Atmosphere (MBTH-Colorimetric Method Applications to Other Aldehydes)." Health Lab. Sci., 7, 173 (1970).

71. M. W. Scoggins, "Spectrophotometric Determination of Ethylene Glycol in Motor Oil." Anal. Chem., 49, 582 (1977).

72. E. Sawicki, T. R. Hauser, T. W. Stanley, and W. Elbert, "The 3-Methyl-2-benzothiazolene Hydrazone Test. Sensitive New Methods for the Detection, Rapid Estimation, and Determination of Aliphatic Aldehydes." Anal. Chem., 33, 93 (1961).

73. E. Sawicki and T. R. Hauser, "Spot Test Detection and Colorimetric Determination of Aliphatic Aldehydes with 2-Hydrazinobenzothiazole. Application to Air Pollution." Anal. Chem., 32, 1434 (1960).

74. B. W. Bailey and J. M. Rankin, "New Spectrophotometric Method for Determination of Formaldehyde." Anal. Chem., 43, 782 (1971).

75. K. J. Bombaush and W. C. Bull, "Gas Chromatographic Determination of Formaldehyde in Solution and High Purity Gas." Anal. Chem., 34, 1237 (1962).

76. R. S. Mann and K. W. Hahn, "Gas Chromatographic Determination of Formaldehyde by Sucrose Octa-Acetate-Columpak T." Anal. Chem., 39, 1314 (1967).

77. R. V. Nauman, P. W. West, and F. Tron, "A Spectrophotometric Study of the Schiff Reaction as Applied to the Quantitative Determination of Sulfur Dioxide." Anal. Chem., 32, 1307 (1960).

78. J. F. Walker, "Formaldehyde." 3rd Ed., A. C. S. Monograph Series, Reinhold Pub., Corp., N.Y., N.Y., 1964, p. 486-8.

79. F. P. Searingelli, B. E. Saltzman, and S. A. Frey, "Spectrophotometric Determination of Atmospheric Sulfur Dioxide." Anal. Chem., 39, 1709 (1967).

80. F. T. Weiss, "Chemical Analysis, Vol. 28, Determination of Organic Compounds, Methods,and Procedures." Wiley-Interscience, N.Y., N.Y., 1970, p. 103.

81. T. R. Hauser and D. W. Bradley, "Specific Spectrophotometric Determination of Ozone in the Atmosphere Using 1,2-Di-(4-pyridyl) ethylene." Anal. Chem., 38, 1529 (1966).

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EXAMINATION AND THESIS REPORT

Candidate: Randle Scott Collard

Major Field: Analytical Chemistry

Title of Thesis: I. A Specific Spectrophotometric Determination of Long Chain Nitro Compounds.
II. A Specific Spectrophotometric Determination of Ozone in Air in the Presence of Nitrogen Oxides.

Approved:

William A. Mayer
Major Professor and Chairman

Carolyn W. Haygood
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Philip W. West

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Date of Examination:

December 4, 1978